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Complementary water-use strategies mitigate drought impacts of mixed arbor-shrub stands in a loess gully area: A quantitative analysis

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Abstract

Aims The loess gully area is a key zone for large-scale revegetation programs in China; however, accurate prediction of mixed arbor-shrub stands responses to variable drought intensities remains poorly quantified. Therefore, quantitative understanding water use strategies of typical revegetation stands under drought is crucial.

Methods Here, we quantitatively investigated the seasonal water use strategies of mixed *Caragana korshinskii* Kom. and *Artemisia vulgaris* Lam. in loess gully region during the growing season in 2023, basing on continuous monitoring of transpiration and 20 times sampling of $\delta^2\text{H}$ and $\delta^{18}\text{O}$.

Results The transpiration of *A. vulgaris* declined significantly in response to a summer drought by 19.7%, whereas that of *C. korshinskii* remained stable, indicating different drought resistance and resilience of these two species. Corresponding to different changes in transpiration, the two species also exhibited divergent water use strategies. In the wet spring, both species took up water predominantly from the surface layer (0–20 cm) (approximately 32.55% for *C. korshinskii* and 51.18% for *A. vulgaris*). However, under the summer drought, *C. korshinskii* switched to soil water of the deep layer (60–110 cm, by 43.23%), whereas *A. vulgaris* mainly relied on soil water of the

middle layer (20-60 cm, by 38.07%).

Conclusion The ability to switch to different water sources indicated that the mixed arbor and shrub stands could reduce interspecific stress and improved drought resistance and resilience. This study enhances our understanding of species-specific water-use strategies in mixed arbor–shrub stands under summer drought, and provides hydrological insights for water resource management in water-limited areas.

Introduction

Global warming has increased the intensity and frequency of drought events and are projected to increase in future (Bello et al., 2019; Allen et al., 2010). Drought stress leads to decreasing soil water availability and increasing atmospheric evaporative demand, which will have adverse effects on plant growth, plant productivity and biodiversity of plantations ecosystem (Manrique-Alba et al., 2022; Ciais et al., 2005; McDowell et al., 2020). Tree mixtures have been identified as an effective strategy to cope with global climate change (Forrester et al., 2016). Mixed plantation species could optimize water use strategies through interspecific niche complementarity and regulate their own physiological processes (e.g., root biomass adjustment, shift in root water uptake depth, stomatal regulation) to sustain transpiration under water resource shortages (Granda et al., 2022; Rodríguez-Robles et al., 2020; Bello et al. 2019; Illuminati et al., 2022).

The Loess Plateau of China has been one of the most intensively restored semi-arid regions globally undergone large-scale revegetation under the “Grain for Green Project” (GFGP) (Fu et al., 2017), abundant cropland area has been converted for plantation forests (Che et al., 2022). Mixed plantation forests, particularly arbors mixed with shrubs, were dominantly reforestation strategy in loess plateau (Che et al., 2023). However, the persistence and stability of revegetation in Loess Plateau of China is often questioned because of water-limits, particularly under droughts of increasing frequency and severity, which have a great impact on vegetation restoration in semi-arid ecosystems by reducing water availability to plants (Gagne et al., 2020). Additionally, planted stands have a relatively simple stand structure and species composition compared with natural stands, which are expected to show higher sensitivity to climate change (Navarro-Cerrillo et al., 2020; Camarero et al., 2021). Thus, research on the dynamics water use patterns of mixed trees on the Loess Plateau is crucial for sustainable water resources management under climate change.

Stable isotopes of hydrogen and oxygen have been widely utilized as a natural tracer in the studies of plant water use sources and absorption proportions (Penna et al., 2018; Brooks et al., 2010), but predominantly provide relative water uptake proportions and often lack absolute water uptake quantification (Brinkmann et al., 2019). Moreover, many previous studies are usually based on data coarser event sampling frequency (Dai et al., 2023), which makes it difficult to obtain a detailed view of threshold and abrupt response mechanisms to drought. In forest ecosystems, sap flux (SF) monitoring offers a direct and dynamic measure of plant transpiration and hydraulic conductance, reflecting real-time responses to environmental conditions (He et al., 2020). Recent research highlights the potential of combining xylem isotope composition and sap flux measurements to quantitatively reveal species-specific water use strategies (Sohel et al., 2023). Yet, integration of isotope data and transpiration fluxes remains incomplete, limiting comprehensive understanding of complex plant water use strategies in response to drought.

C. korshinskii and *A. vulgaris* are two widely distributed afforestation species planted in mixed stands in the loess gully area of southern Ningxia to prevent soil erosion and control desertification (Yang et al., 2021). However, the drought resistance of afforestation species requires further research, particularly in semi-arid regions where less soil moisture and precipitation are the only water sources with fragile ecosystem. This study combined stable isotope and sap flow methods to investigate the interspecies water use patterns and determine the relationship with environmental variables. Our aim was to: (1) identify the seasonal variation of transpiration of restored plantation and to determine the effects of environmental factors; (2) analyze the stable isotopic characteristics of precipitation, soil water, and xylem water of restored plantations; (3) quantitatively explore the contribution of potential water sources and coexistence strategies of arbor-shrub mixed stands under seasonal drought conditions.

Materials and methods

Study site

The study site is located in the Hechuan watershed, south of Ningxia, western loess plateau of China (35°59' N, 106°27' E) (Fig.1). The study site belongs to a semi-arid and sub-humid climate zone with the average annual temperature of 7.28 °C and annual precipitation of 461.9 mm from 1960 to 2019, the precipitation of this region in summer (June-August) accounting for approximately 56.5% of annual precipitation. The area is in an interlaced zone that experiences wind and water erosion and constitutes the transition from the hilly loess area to Mu Us Desert. The study area is a typical area of serious soil erosion in the Loess Plateau of China, soil erosion modulus was $1200 \text{ t km}^{-2} \text{ a}^{-1}$, which led to the broken topography here (Wang et al., 2024). The topography here has been greatly reshaped by serious soil erosion during the past thousand years and deeply eroded gullies existed around. The soil texture was silty loam (USDA classification), and the granular composition was 32.9% sand, 65.5% silt, and 1.6% clay.

As the core region of the GFGP in the western China, large-scale level arable lands were transformed to woodlands on upland hillslopes. Artificial vegetation in the region such as Korshinsk pea (*Caragana korshinskii* Kom.) and Apricot (*Armeniaca vulgaris* Lam., cv. 'Hongmeixing') are the main revegetation plants. To improve the survival of revegetation, engineering measures are usually combined to afforestation at the early stage of slope afforestation (Wang et al., 2016). Consequently, the combined pattern of mixed *C. korshinskii* and *A. vulgaris* in the level ditch was typically constructed in this region in 2002. The *A. vulgaris* are generally planted in the ditch. The *C. korshinskii* are generally planted in the ridge of the level ditch to protect it from water erosion and extend its service time. The average height and diameter at breast height was shown in Tab.1. The water table is about 20 m below the land surface, resulting in soil water available to plants being solely recharged by rainwater (Wang et al., 2020).

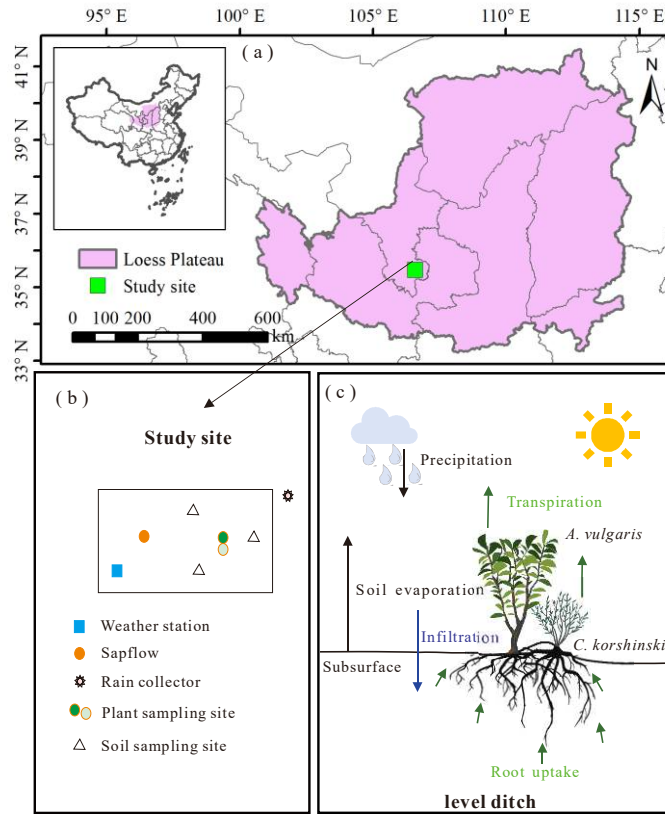


Fig.1. Location of study site on the Loess Plateau in China (a), the schematic view of experimental station (b), the schematic profile view of level ditch (c).

Tab.1. Basic characteristics of sample vegetation

Tree species	Tree height(m)	Crown area (m ²)	Basal diameter (BD) (cm)	Sapwood area (As) (cm ²)	Age
<i>A. vulgaris</i>	5.55±0.87	20.86±9.23	49.15±22.74	$A_s = 0.0208 BD^{2.0173}$ $R^2 = 0.9968$	21 y
<i>C. korshinskii</i>	2.41±0.33	10.76±3.22	6.31±0.99	$A_s = 0.0297 BD^{1.8859}$ $R^2 = 0.9973$	21 y

Monitoring of meteorological data

Precipitation (P), air temperature (T_a), relative humidity (RH), and mean solar radiation (R_s) were measured every 5 min using a weather station (HCD6818N) about 10 m away from the sampling plot (Fig.1). The precipitation (P) amount on any rainy day were also recorded by rain gauge (RS-YL-PL-4-02) with 0.2 mm resolution. Vapor pressure deficit (VPD, kPa) was obtained from empirical equations for T_a and RH (Hayat et al., 2020):

$$VPD = 0.611 \times \exp\left(\frac{17.27T_a}{237.3 + T_a}\right) \times (1 - RH) \quad (1)$$

Measurements of sap flow

Three typical *A. vulgaris* and *C. korshinskii* stands were selected in the standard plots for the continuous monitoring of sap flow from April to October 2023, using thermal dissipation probes (STDP, Sinton Technology Co., Ltd, China) and Remote Terminal Unit (Blueland Scientific Co., Ltd, China) recorded data at 5 min intervals. To avoid the thermal effect caused by direct sunlight, thermal dissipation probes wrapped in aluminum foil were installed in the trunks at breast height for *A. vulgaris* and at the main basal root for *C. korshinskii*. To avoid damage to sample trees, increment cores were extracted from neighboring individuals of the same species using an increment borer. A regression relationship between sapwood area and diameter at breast height (DBH) was established and subsequently applied to estimate the sapwood area of the target sample trees as Tab.1. The thermal dissipation probes consisted of upper and lower probes containing a copper-constantan thermos-couple were used for heating. The temperature difference (ΔT , °C) between the two probes and the maximum ΔT (ΔT_m , °C) (when the sap flow was near zero) were calculated, and the sap flow velocity (V , $\text{cm}^3 \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) was calculated according to the empirical calibration formula proposed by (Granier 1987):

$$V = 0.0119 \left(\frac{\Delta T_{\max} - \Delta T}{\Delta T} \right)^{1.231} \quad (2)$$

where V is the sap flow density ($\text{cm}^3 \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$), $\Delta T_{\max}$ (°C) represents the maximum ΔT observed during a day or an extended period, typically indicating a little flow condition.

Individual plant water consumption (E_h , $\text{L} \cdot \text{d}^{-1}$) of *A. vulgaris* and *C. korshinskii* was calculated using the following formula:

$$E_h = \frac{V \times A_s \times 3600 \times 24}{1000} \quad (3)$$

where A_s is the total sapwood area of the tree (cm^2). Based on sapwood area investigation using a tree growth cone, the relationship between the Basal diameter (BH) and the A_s was established (Tab.1).

Additionally, to upscale SF to canopy transpiration, transpiration rates (T_r , mm) of *A. vulgaris* and *C. korshinskii* were calculated by E_h followed:

$$T_r = \frac{E_h}{A_c} \quad (4)$$

where A_c is the effective canopy area (m^2).

Sample collection

Soil and plant samples were collected 2 to 3 times per month during study period (7.4.2023-31.10.2023) for a total of 20 times. To avoid precipitation disturbance, samples were generally collected within 3–10 days after precipitation. *C. korshinskii* and *A. vulgaris* which grow well and are distributed evenly were selected to collect their mature branches. The branches were divided into 5-7 cm segments and the bark was removed quickly. At the same time, 5-10 mature leaves of

the trees were sampled. To prevent water loss caused by distillation, the leaves and branches were quickly placed in the 12 mL glass bottles and sealed with Parafilm immediately. Three soil profiles randomly around the sample plants within the plot were selected to collect soil water samples (Fig.1b) at depths of depths of 0-20, 20-40, 40-60, 60-80 and 80-110 cm from the soil layers by soil auger. The fresh soil sample were mixed and separated into two parts: one was placed in the 12 mL glass bottle and sealed with Parafilm for subsequent water isotope analysis and the other was placed in the aluminum box for soil water content measurement.

Precipitation $\geq 5\text{mm}$ were collected in an open area after each precipitation event during study period. There are no tall trees or buildings affecting incoming rain. At this site, a polyethylene bucket was placed with a plastic funnel in, and a Ping-Pong ball was placed in the funnel to prevent evaporation and fractionation of the water in the bucket. We collected a total of 34 precipitation samples during study period, put each sample into a 100 mL polyethylene bottle with no headspace left, and immediately sealed the bottle with Parafilm.

All samples were brought back to the laboratory and put in the refrigerator ($\sim 20^\circ\text{C}$) to prevent water evaporation until experimental analysis. The water of xylem, leaves and soil samples was extracted by an automated cryogenic vacuum distillation system (LI-2100, LICA Inc, China) for stable isotopic analysis. The system adopts an auto ultra-low pressure and temperature condensation method. The water in the sample is distilled and condensed by the auto ultra-low pressure system and the cooling system to acquire the extracted water successfully without fractionation. The extracted water was filtered by a $0.22\ \mu\text{m}$ organic filter membrane to remove organic contamination, and then was stored in the refrigerator ($4\ ^\circ\text{C}$) until analysis. Comparison with the oven-drying method revealed that the water extraction rate of these samples was $> 98\%$. Soil water content (SWC) was calculated by weighing soil samples before and after drying them at 105°C for 24 h.

Isotopic analysis

The analysis of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of all the above water samples was completed using a liquid-water isotope analyzer (DLT-100, Los Gatos Research, USA) in the stable isotope laboratory of Institute of Geographic Sciences and Natural Resources Research. The remainders were processed and checked for spectral interferences (caused by potentially coextracted organics such as methanol or ethanol) using the Spectral Contamination Identifier post-processing software (LWIA-SCI, Los Gatos Research, Inc.). Only results showing ‘norm’ were further used. The measurement results are expressed in terms of the conventional deviation value (δ -value) relative to the Vienna Standard Mean Ocean Water (V-SMOW) :

$$\delta(\%) = [R_{\text{sample}} - R_{\text{standard}} / R_{\text{standard}}] \times 10^3 \quad (5)$$

where R_{sample} and R_{standard} are isotopic ratios ($^2\text{H}/\text{H}$ or $^{18}\text{O}/^{16}\text{O}$) in the samples and references, respectively. The precision of the Analyser is $\delta^2\text{H} < 0.1\ \text{‰}$ and $\delta^{18}\text{O} < 0.01\text{‰}$, respectively.

Line-conditioned excess (lc-excess) was calculated for local evaporative effects. The lc-excess can characterize the evaporation index of different water bodies relative to the local precipitation (Landwehr et al., 2014):

$$lc - excess = \delta^2H - a \times \delta^{18}O - b \quad (6)$$

where a and b are the slope and intercept of the local meteoric water line (LMWL), respectively, and δ^2H and $\delta^{18}O$ are the isotopic values of hydrogen and oxygen in the different water bodies. The physical meaning of lc -excess is expressed as the degree of deviation of the isotope value in the sample from LMWL, indicating the nonequilibrium dynamic fractionation process caused by evaporation. Generally, the change in lc -excess in local precipitation is mainly affected by different water vapor sources, and the annual average is 0. Since the stable isotopes in soil water are enriched by evaporation, the average lc -excess is usually negative (Sprenger et al., 2017).

Potential water source partitioning

A Bayesian mixture model (MixSIAR 3.1.12) was used to calculate the relative proportion of plant water uptake to each potential water source (Stock et al., 2018), since the model considered the variability and isotopic fractionation of the isotopic signal of plant xylem water and potential water sources. Since the farmers did not irrigate the plants in the experimental site and the water table within the stands estimated from the groundwater well was > 20 m, the source of water for plants was soil water. For the subsequent analysis and comparison, the plant water sources were divided into the shallow layers (0–20 cm), middle layers (20–60 cm), deep layers (60–110 cm) according to the variability in isotopic compositions and SWC of the soil water. First, the raw isotope compositions (δ^2H and $\delta^{18}O$) of xylem water for each plant (*A. vulgaris*, *C. korshinskii*) and soil water for three layers were used as mixture data and source data to upload to the MixSIAR model. Second, the discrimination data was set to zero for both δ^2H and $\delta^{18}O$ since the isotope fractionation was supposed to not happen during the process of plant water uptake (Dawson and Ehleringer 1991). Third, the model was set to run with ‘very long’ or ‘extreme’ with tuning lengths. Gelman-Rubin and Geweke diagnostic tests were employed to judge whether the model reach to convergence before accepting the output of MixSIAR. The absolute water uptake (AWU) for each species was calculated by scaling E_h to RWU for the corresponding soil layers.

The proportional similarity index (PSI) was used to evaluate the water uptake overlap between *C. korshinskii* and *A. vulgaris*, which was calculated as follows (Chen et al., 2023; Hoekstra et al., 2014):

$$PSI = 1 - 0.5 \sum_{i=1}^n |P_{Ci} - P_{Ai}| \quad (7)$$

Where P_{Ci} and P_{Ai} represent the proportional uptakes of i water source by *C. korshinskii* and *A. vulgaris*, respectively. When PSI equals zero, it indicates *C. korshinskii* and *A. vulgaris* do not share water sources, and when PSI equals one, it means the water sources of two species overlapped completely.

Statistical analyses

The relative extractable soil water (REW) was calculated to reflect soil moisture conditions as follows:

$$REW = \frac{SWC - SWC_{\min}}{SWC_{\max} - SWC_{\min}} \quad (8)$$

where $SWC_{\max}$ and $SWC_{\min}$ are the maximum and minimum SWC at each soil depth across the study period. When the REW drops below 0.4, the vegetation is assumed to be suffering from dry conditions (Zhou et al., 2013; Granier et al., 2007).

Resistance, resilience, and recovery to drought were expressed as ratios of Js at the end of the drought to those measured at the beginning of the drought (Iqbal et al., 2021; Lloret et al., 2011)

$$Resistance\ Index = \frac{E_{h(t)}}{E_{h(t-1)}} \quad (9)$$

$$Resilience\ Index = \frac{E_{h(t+1)}}{E_{h(t-1)}} \quad (10)$$

$$Recovery\ Index = \frac{E_{h(t+1)}}{E_{h(t)}} \quad (11)$$

Here, $E_{h(t)}$ is the daily sap flow rate ($kg\ d^{-1}$) during the drought period “t”, $E_{h(t-1)}$ is the average sap flow rate three days preceding drought (i.e., when $REW < 0.4$), and $E_{h(t+1)}$ is the average sap flow rate three days following drought. Ratios close to 1.0 imply greater resistance and resilience to drought, and capacity to recover from drought.

One-way analysis of variance (ANOVA) with Duncan’s honestly significant difference (HSD) post-hoc tests was performed to examine differences in soil water content, soil water *isotope*, and plant water isotope. The Pearson’s rank correlation test was used in the correlation analysis to clarify the relationship between environmental factors and the transpiration of *A. vulgaris* and *C. korshinskii* in different season, with significant levels of 0.05, 0.01, and 0.001. All statistical analyses were performed in SPSS 26.0 (SPSS Inc., Chicago, IL, USA).

Results

Meteorological conditions and soil water content

The total precipitation was 298.2 mm during study period, while long-term average was 432.2 mm (1960-2019), which was found to be a very dry year based on the P-III frequency distribution curve of long term precipitation at Guyuan station. The mean T_a from June to October averaged $14.68 \pm 6.08\ ^\circ C$, the average VPD during the study period was $0.44 \pm 0.41\ kPa$, the RH averaged $78.55 \pm 16.35\%$, the daily mean R_s was $120.74 \pm 104.50\ W \cdot m^{-2}$, the ET_p averaged $6.44 \pm 2.88\ mm/d$ during study period. Compared to long term meteorological conditions, the study period of 2023 lasted through a very dry and warm summer (June-August) (Fig.2b). Precipitation was rare in summer, with most summer rainfall events being $< 5\ mm$, with the lowest precipitation occurring in June at 19.8 mm, 66% less than the multi-year monthly average for that month (57.82 mm). Most rainfall events occurred mainly in early August with 53.6 mm, but also much less than multi-year monthly average for that month (110.42 mm). Seasonal variation patterns of meteorological factors (T_a , VPD, R_s , LI and ET_p) were identical, peaking in June and July, and T_a averaged 17.66 and

19.60 °C on June and July, respectively, and VPD exceeded 1 kPa most of the summer, while the relative humidity was low (mean 66.03%) (Fig.2c).

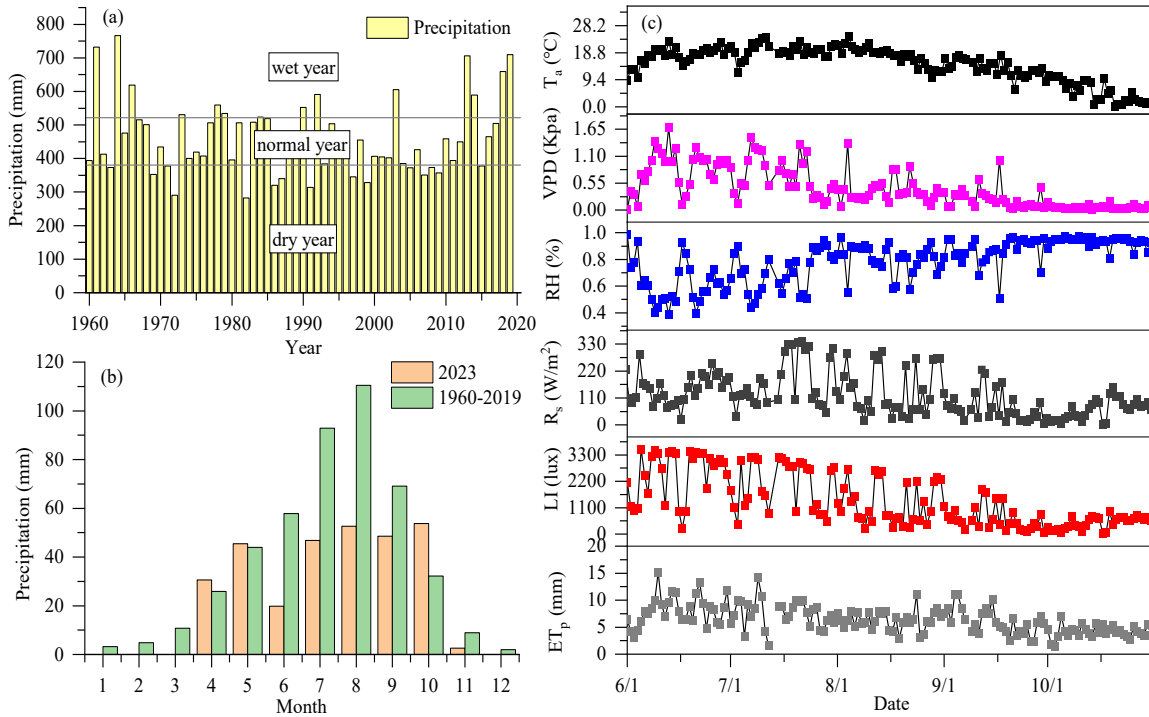


Fig.2. Precipitation variation at Guyuan station from 1960 to 2019 (a), Monthly precipitation of study site (b), Time series of daily hydroclimate variables (c): air temperature (T_a), vapour pressure deficit (VPD), relative humidity (RH), solar radiation (R_s), light intensity (LI), the potential evapotranspiration (ET_p) from June to October in 2023.

The SWCs in the 0–110 cm soil layer during the study period ranged from 4.15% to 17.85%, with average of $7.40 \pm 3.00\%$ (Fig.3a). With increasing depths, the SWCs and seasonal variabilities showed a decreasing trend, with the highest value and greatest range at 0–20 cm and the lowest value and smallest range at 60–80 cm, averaged $9.70 \pm 4.20\%$ and $6.26 \pm 0.91\%$, respectively. The SWCs in shallow layer (0–20 cm) responded to precipitation rapidly, and fluctuated more significantly with arising precipitation than other layers (20–110 cm) that only increased with larger precipitation events in late summer, but then declining rapidly as soon as precipitation decreased, reflecting the low soil water retention. SWCs values were generally relatively low and very dry ($<10\%$) throughout most of the summer until mid-August when they began to re-wet in surface layer (Fig.3a). The REW is an index that represents the soil water available for plants. REW values in the 0–100 cm soil layer ranged from 0.0626 to 0.2413 (Fig.3b) from mid-June to August. Although precipitation and SWCs began to re-wet in August, the REW middle and deep layer still at low value ($REW < 0.4$), indicating periods of soil drought. We thus defined the period between 5 June 2023 and 23 July 2023 as a drought period (indicated by the grey shaded areas in Figs 3, 6, 7). Overall, these data indicate that the hydrometeorological conditions were warmer and drier in summer, with higher evaporative demands. Summer drought led to a decrease in SWCs, the SWCs of the entire soil profile was at low values from June to August, and the average SWC ($\pm 1SD$) from 0 to 100 cm during this period is $6.54 \pm 2.49\%$, lower than that in other periods of $8.07 \pm 3.27\%$ for autumn and $8.11 \pm 2.07\%$ for spring.

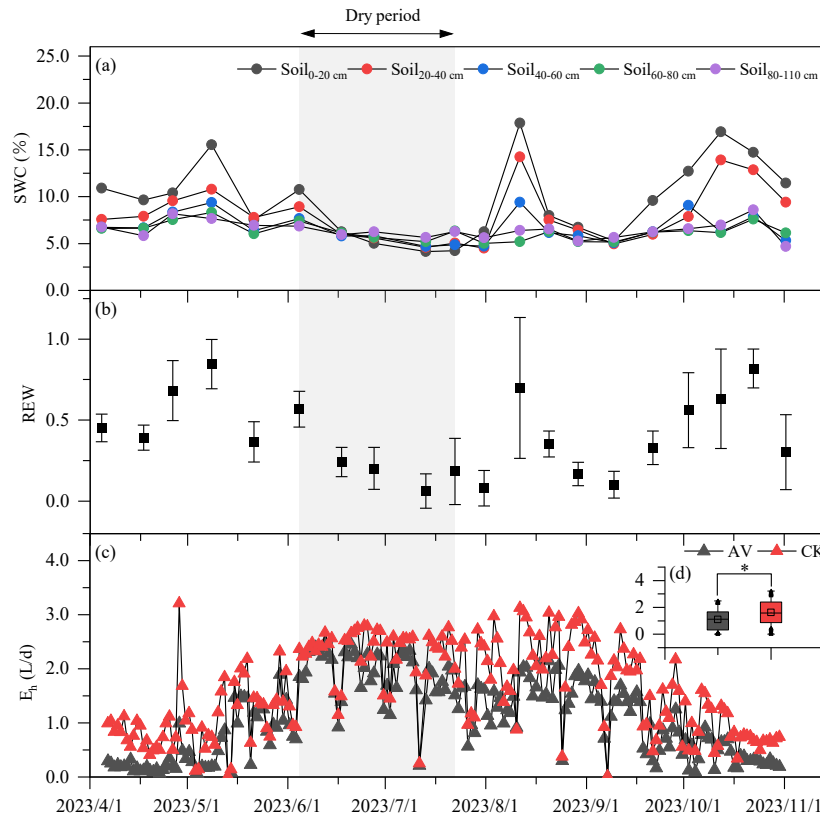


Fig.3. Soil water content (SWC) at different depths (a), relative extractable water (REW) (b), daily transpiration (E_h) of *A. vulgaris* and *C. korshinskii* (c), boxplots for total transpiration (E_h) of *A. vulgaris* and *C. korshinskii* (d) during the observation period (April to October 2023). An asterisk indicates significant differences ($P < 0.05$). The grey shaded area depicts the drought period.

Plant transpiration dynamics

The E_h of *A. vulgaris* was significantly lower than the *C. korshinskii* ($p < 0.05$) (Fig.3c), varied from 0.008-2.484, 0.009-3.207 $L \cdot d^{-1}$ with an average of 1.098 ± 0.736 , $1.626 \pm 0.831 L \cdot d^{-1}$. The E_h of *A. vulgaris* and *C. korshinskii* with normal growth was significantly different among three seasons in 2023 ($p < 0.05$). E_h of two plants increased from April to May, with increasing by 19.8% and 68.0% of *C. korshinskii* and *A. vulgaris*. Drought reduced E_h differently in summer and autumn for the two species (Fig.3c). E_h of *A. vulgaris* decreased for long time from late June to August when drought was most extreme, while *C. korshinskii* keep stable until autumn, with drops of 19.7% for *A. vulgaris* and increase of 3.1% for *C. korshinskii*, indicating *C. korshinskii* affected little by drought. Sap flow rates of two plants exhibited declining trends in autumn (September-October), maybe affected by the leaf senescence. But they increased briefly during larger rainfall events. The resistance, resilience, and recovery indices significantly differed between the two specie. The resistance, resilience, and recovery were least for *A. vulgaris*, with average of 0.60, 0.26 and 0.68, and greatest for *C. korshinskii* with average of 0.79, 0.42 and 0.69. As *C. korshinskii* provided resistance and recovery values close to 1.0, it was more resistant to drought than *A. vulgaris*.

In the Pearson rank correlation analysis of daily values, meteorological conditions (P , LI , R_s , ET_p , T_a , RH , VPD), REW , E_h of summer and autumn were combined respectively. The regression

analysis revealed a moderately (for *C. korshinskii*) and strong (for *A. vulgaris*) negative correlation between transpiration of trees and REW of shallow layer in Autumn ($p \leq 0.05$). However, transpiration of *C. korshinskii* negatively correlated with REW of all soil profile, and transpiration of *A. vulgaris* positively correlated with REW of all soil profile in Summer. Precipitation simply negatively correlated with shallow soil REW in summer, but showed moderately negative correlations with daily transpiration of three trees in both season ($p \leq 0.05$). E_h was positively correlated with LI , R_s , ET_p , T_a and VPD ($p \leq 0.05$), but it was negatively correlated with RH ($p \leq 0.05$) in both two season.

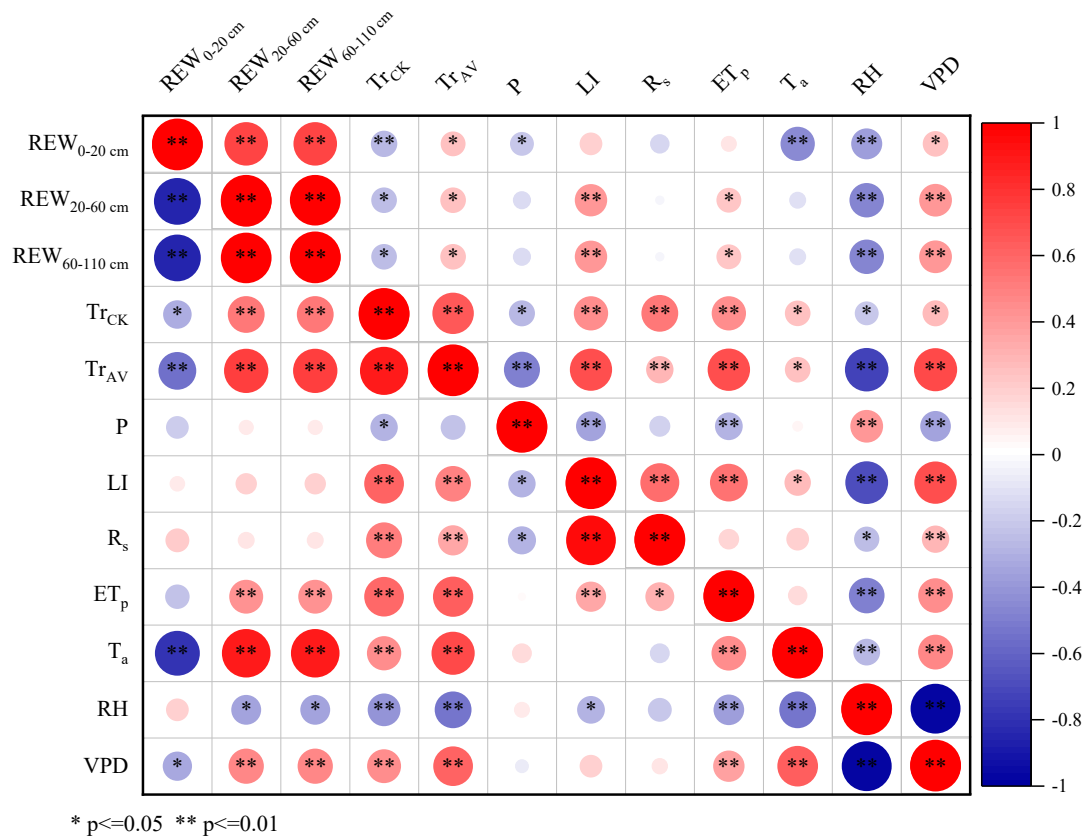


Fig.4. Pearson rank correlation between daily transpiration of *C. korshinskii* and *A. vulgaris* and environmental factors during summer (upper right) and during autumn (lower left) seasons

Isotopic patterns in soil and plant water pool

The local meteoric water line was calculated by the regression line between δ^2H and $\delta^{18}O$ in local precipitation (LMWL: $\delta^2H = 7.82\delta^{18}O + 11.38$ ($R^2 = 0.98$)) (Fig.5). The δ^2H and $\delta^{18}O$ value of precipitation ranged from -115.8‰ to -3.2‰ and from -16.44‰ to -1.87‰, and the average was -56.17‰ and -8.64‰. The change in isotope of precipitation during the complete sampling period was affected by precipitation amount, the precipitation in June was almost the most enriched; the weighted δ^2H and $\delta^{18}O$ were -23.6‰ and -4.70‰, respectively. The most depleted precipitation occurred in October, with the weighted δ^2H and $\delta^{18}O$ being -92.7‰ and -13.66‰, respectively.

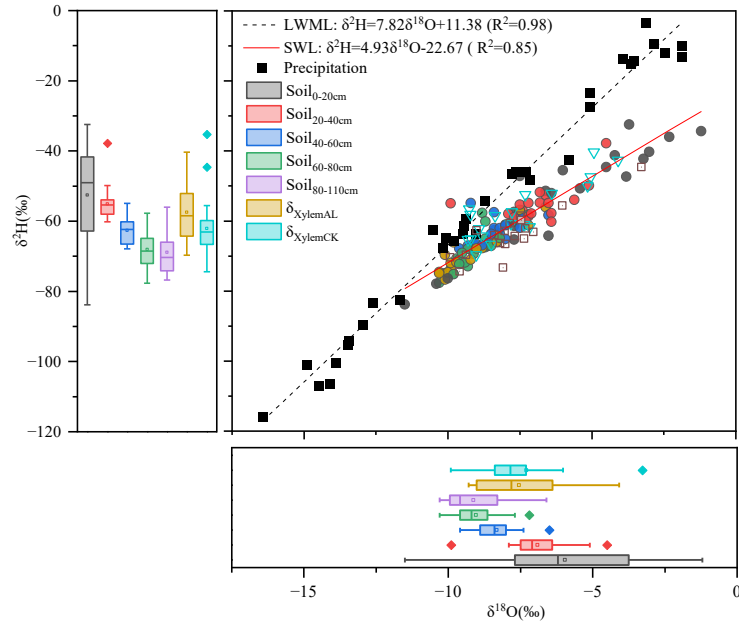


Fig.5. Scatter distribution of $\delta^2\text{H}$ - $\delta^{18}\text{O}$ soil water, and xylem water of different plants

The statistical analysis of $\delta^2\text{H}$, $\delta^{18}\text{O}$ of precipitation, soil water and plant water was shown in table 1. The isotopic values of the soil water were relatively richer than precipitation (-61.5‰ , δD ; -7.88‰ , $\delta^{18}\text{O}$; -11.25‰ , Lc-excess). Additionally, a linear relationship of $\delta^2\text{H}$ - $\delta^{18}\text{O}$ for soil water (SWL) was observed (Fig.5), as expressed by the equation: $\delta\text{D} = 4.93\delta^{18}\text{O} - 22.69$ ($R^2 = 0.85$). The mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in the xylem water of *C. korshinskii* and *A. vulgaris* was -62.1‰ and -57.5‰ , -7.29‰ and -7.56‰ , respectively. The mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in leaf water of *C. korshinskii* and *A. vulgaris* was -25.4‰ and -19.3‰ , 0.75‰ and 3.42‰ , respectively, and none of them are significantly different ($p > 0.05$). The space between the LMWL and SWL is defined as the soil-xylem mixing space (Evaristo et al., 2017), while most of the xylem samples were outside the distribution mixing areas (Fig.5). Furthermore, the δxyl values of plant water closely resemble the isotopic characteristics of deeper soil water, suggesting that these water sources serve as the primary contributors to root water uptake.

Tab.2. The statistics analysis of isotope composition

		Average	Stev	Min	Max
Precipitation	δD	-56.2	33.6	-115.8	-3.2
	$\delta^{18}\text{O}$	-8.64	4.26	-16.44	-1.87
	Lc-excess	0.01	4.48	-9.89	9.67
Soil water	δD	-61.5	10.4	-83.8	-32.4
	$\delta^{18}\text{O}$	-7.88	1.94	-11.52	-1.24
	Lc-excess	-11.25	6.94	-36.08	10.91
δXylem_{AV}	δD	-57.5	8.1	-69.7	-40.3
	$\delta^{18}\text{O}$	-7.56	1.69	-9.30	-4.09
	Lc-excess	-9.78	7.39	-22.14	4.43

δXylem_{CK}	δD	-62.1	9.3	-74.4	-35.3
	$\delta^{18}\text{O}$	-7.29	2.57	-9.92	1.50
	Lc-excess	-16.42	11.97	-58.49	-3.65
δLeaf_{AV}	δD	-19.3	26.7	-64.9	29.1
	$\delta^{18}\text{O}$	3.43	7.84	-7.02	18.54
	Lc-excess	-57.47	38.80	-133.63	-3.99
δLeaf_{CK}	δD	-25.4	26.2	-74.6	7.9
	$\delta^{18}\text{O}$	0.75	6.51	-8.35	10.51
	Lc-excess	-42.63	28.28	-87.33	-4.26

During the study period, the surface soil layer (0–20 cm) exhibited relatively enriched isotopic values ($\delta^2\text{H}$: -52.6‰; $\delta^{18}\text{O}$: -5.97‰; lc-excess: -17.27‰), whereas the middle (20–60 cm) and deep soil layers (60–110 cm) showed more depleted values, recording $\delta^2\text{H}$ at -58.9‰ and -68.5‰ and $\delta^{18}\text{O}$ at -7.62‰ and -9.08‰ respectively. This indicates a trend of isotopic depletion with depth, accompanied by a reduction in the range of variation, suggesting a progressive stabilization of soil water isotopes at deeper depths. These isotopic patterns reflect the combined influences of precipitation infiltration and evaporative enrichment. Higher $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in the surface soil suggest significant evaporative fractionation, whereas the more depleted isotopic composition in deeper layers primarily represents infiltrated precipitation with minimal evaporative influence. The range of soil water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ narrowed with increasing depths (Figs 6d, e). At surface layer (0–20 cm), the maximum and minimum isotope values were recorded on June and on October, respectively, while the peak values of soil water isotopes in other layers appeared later and became unclear with increasing depth, indicating lag effect among different soil layers in water infiltration and recharge. The lower lc-excess values for surface soil water also appear in the drought period. Except for surface soil water, seasonal variation in lc-excess of other soil layer and xylem water were not obvious (Figs 6g–i).

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the two plants exhibited similar temporal variation patterns (Figs 6b, e), with the maximum values occurring in April and the minimum values in June–July. At the beginning of the study, all vegetations exhibited enriched heavy isotope signatures, with *C. korshinskii* showing a more pronounced effect in early April. After June, heavy isotopes in xylem water were gradually depleted, reaching their lowest levels by mid-July. However, δxyl became more enriched again in rewetting period (early August), opposite to precipitation and soil water isotopic composition, and remained relatively stable until October.

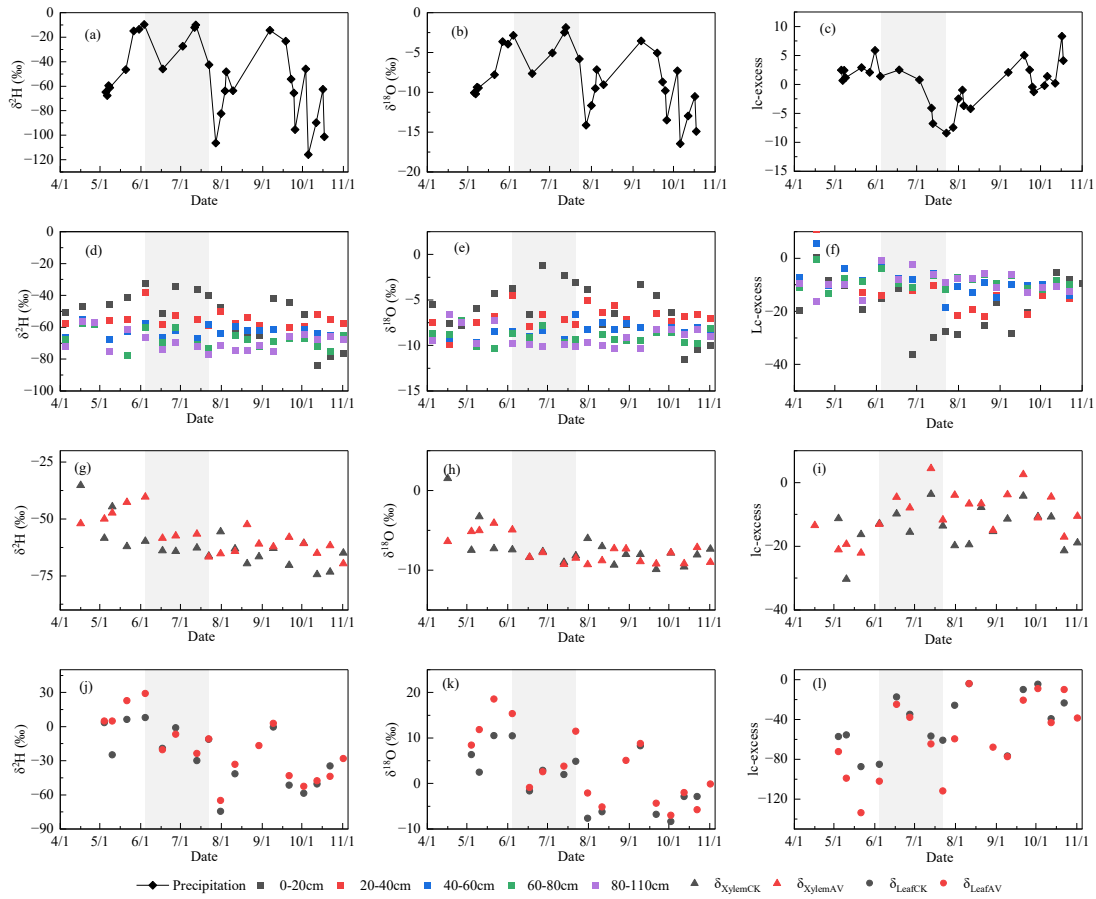


Fig.6. Temporal variations of $\delta^2\text{H}$, $\delta^{18}\text{O}$, and lc-excess of precipitation (a, b, c), soil water (d, e, f), and xylem water (g, h, i), leaf water (j, k, l) of *A. vulgaris* and *C. korshinskii* during study period. The grey shaded area depicts the drought period.

Variations of plant water uptake

Combining the characteristics of meteorological conditions and SWCs in the sample plots, the June to August 2023 was defined as a summer drought period (indicated by gray shading in Figs 3, 6, 7) in this study, to further explore plant water use in the under different moisture conditions. The water use pattern of *A. vulgaris* and *C. korshinskii* were significantly different seasonally (Fig.7). In spring, 0–20 cm soil water was sufficient, which caused the *C. korshinskii* and *A. vulgaris* to take up a relatively high proportion of 0–20 cm soil water during April–May, with an average water uptake proportion of 32.55% and 51.18%. Once entering summer, the uptake proportion of surface soil water by *C. korshinskii* and *A. vulgaris* decreases significantly, while the uptake proportion of middle and deep soil water both increases somewhat, making the absorption proportion of each water source switched in this period under drought stress. And the main water source of *C. korshinskii* was deep layer water in summer, with an average water uptake proportion of 43.23%, while the main water source of *A. vulgaris* was middle layer water in summer, with an average water uptake proportion of 38.07%. During the drought period, these two mixed trees can make rational use of water resources at different depths, and the mixed forests can coexist reasonably. The results of absolute contribution to total water uptake for two plants demonstrate that these species exhibit adaptive shifts in water uptake depth to cope with seasonal drought stress. During

the summer, *A. vulgaris* and *C. korshinskii* primarily absorbed water from middle layer and deeper soil layer, with average absolute water uptake contribution on 0.1695, 0.2611 mm, respectively.

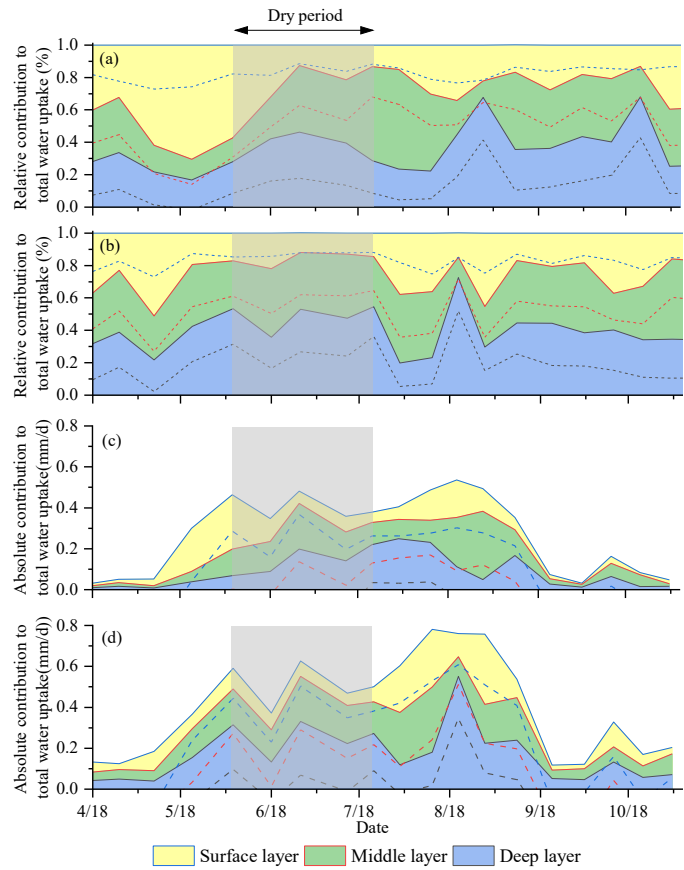


Fig.7. Relative contribution to total water uptake of arbor (*A. vulgaris*) and shrub (*C. korshinskii*) (a, b) and absolute contribution to total water uptake of arbor (*A. vulgaris*) and shrub (*C. korshinskii*) (c, d). The grey shaded area depicts the drought period. Data shown are mean values – SE (dashed lines)

Tab.3 Proportional similarity index (PSI) between *A. vulgaris* and *C. korshinskii*.

Month	4	5	6	7	8	9	10
<i>PS index</i>	0.94	0.69	0.79	0.94	0.60	0.94	0.81

Discussion

Seasonal transpiration dynamics and its response to summer drought

In this study, two mixed plant species exhibited little high transpiration rates throughout the study period compared to similar ecosystems in other regions. Even for the same species, the SF may be largely different under different site conditions (Wang et al. 2020). For instance, Han et al., (2023) demonstrated that *C. korshinskii* consumed 0.6 kg/day water in an arbor-shrub mixed stand with *Elaeagnus angustifolia*. (Wang et al. 2020) demonstrated that *C. korshinskii* consumed 1.6 kg/day water in a *C. korshinskii* stands, which is similar to our study. Although pure plantation usually consumes more water than that in mixed plantation on average (Han et al. 2023). These similarities

can often be related to the amount of precipitation received at our site during study period. Rainfall is the main water resource in this rain fed area. The water table in rain-fed area is often below several dozen meters from the ground surface and continues to lower. Rainfall is the main water resource in this rain-fed area.

The arbor-shrub mixed plantation exhibited pronounced seasonal variations in transpiration dynamics. Transpiration rates were generally higher during the spring and early summer, coinciding with favorable soil moisture conditions and elevated atmospheric demand. As the season progressed into midsummer, prolonged drought led to the change in sap flow and transpiration in both species, followed by partial recovery in autumn when precipitation events increased soil water availability. Moderate reductions in transpiration under water stress are considered as an effective strategy to maintain the water in the plant to promote survival (Garg et al., 2015). Plants usually adjust their physiological activities to survive in response to the external environment fluctuations (Cavanaugh et al., 2011), including air temperature (T_a), vapor pressure deficit (VPD), potential evapotranspiration (ET_p), solar radiation (R_s), light intensity (LI), and soil water content (SWC) (Tie et al., 2017). Soil moisture provided the material basis for water consumption of the two species. The average solar radiation, average temperature, and vapor pressure deficit provide the energy and tension for water consumption (Nöjd et al., 2017). And when soil water is limited, it can modulate the effect of meteorological factors on plant transpiration (Hayat et al. 2020)

While in autumn with good water conditions, soil water was regularly replenished by substantial rainfall at a relatively high frequency, minimizing soil drought stress, *C. korshinskii* and *A. vulgaris* became more sensitive to environmental factors (Jiao et al., 2019). Except for precipitation, the contribution of other explanatory variables to *C. korshinskii* and *A. vulgaris* water consumption in wet autumn was highly statistically significant ($p < 0.01$). Furthermore, we found that that sap flow in two mixed species was significantly negatively correlated with REW of surface layer during wet autumn (Fig.4). Overall, although soil water content (SWC) remained relatively high during autumn, transpiration was weak during these periods. It attributed to the end of the growing season, the physiological development of the plants was slow. Although the soil water content was high, absorption and utilization by plants were low. Concurrently, solar radiation and the vapor pressure deficit decreased, and these factors limited water consumption.

Sap flow rates are not only related to environmental factors, but they are also influenced by species-specific trait (Nadine et al., 2016). In this study, the onset of summer triggered variation of water consumption trends, indicating the adjustment of the plant water use adaptive strategy in the mixed plantation. However, *C. korshinskii* and *A. vulgaris* demonstrated divergent transpiration dynamics in response to drought. Notably, *A. vulgaris* began to decline transpiration under drought, whereas *C. korshinskii* exhibited a state increase until August (Fig.3c), suggesting a delayed or more drought-tolerant strategy. *C. korshinskii* was just significantly related to LI, R_s and ET_p , while *A. vulgaris* was just related to P, LI, ET_p , RH and VPD ($p < 0.01$) (Fig.4). These meteorological factors influence plant water use primarily by modulating stomatal conductance, which is a key determinant of water consumption in forest ecosystems (Zhang et al., 2017). Generally, low soil moisture combined with high temperature can lead to water stress, reducing leaf water potential, suppressing photosynthesis, causing stomatal closure, decreasing sap flow, and ultimately limiting cell expansion and plant growth (Joshi et al., 2016).

The sap flow of plants correlated positively with REW during summer for *A. vulgaris* and exhibited a significant negative correlation for *C. korshinskii*, and the absolute value of the correlation coefficient between transpiration rate (E_h) and REW in deeper soil layers was lower than that for shallower layers (Fig.4), indicating that transpiration is more sensitive to moisture availability in the upper soil layers. One possible explanation for this pattern is that soil water content (SWC) in the deep layers tends to be more stable than in shallow layers, and plants generally prefer to extract water from more stable source (Moreno-Gutiérrez et al., 2012).

Seasonality of ecohydrological fluxes and water cycling of different plants

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of precipitation exhibited enrichment in heavy isotopes during the hot and dry summer and depletion during the wet autumn (Fig.6), a pattern also observed in the Berlin region (Kuhlemann et al., 2021). This isotopic seasonality is globally driven by variations in temperature and precipitation (Bowen 2008). Previous studies have demonstrated that precipitation patterns and vegetation cover influence the amount of water replenishing the soil (Luo et al., 2023; Robertson and Gazis 2006; Yang and Fu 2017). Based on 20 soil water isotope measurements during the observation period, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of shallow soil water showed similar seasonal variations to those of precipitation. However, isotopic fractionation of soil water varies depending on consumption processes such as soil evaporation and plant transpiration. Recent studies suggest that soil water lc-excess is an important indicator of soil evaporative intensity. In this study, lc-excess in soil water remained relatively stable overall (Fig.6), but showed a marked decline in surface soils during the drought period, with values lower than those reported. The minimum lc-excess observed during the drought reached nearly -30‰, significantly lower than the -20‰ in a humid region (Dai et al. 2023). These results suggest that tree-shrub plants in the study area exhibit stronger evaporation of shallow soil water during drought conditions, reflecting species-specific water use strategies in response to extreme climatic stress.

High-frequency isotopic sampling of soil water at multiple depths and xylem water in plants revealed the seasonal dynamics of plant water uptake sources. The results demonstrated pronounced ecohydrological shifts in tree water use during the early growing season, particularly after leaf emergence in spring. In April and May, xylem water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ (δ_{xylem}) values exhibited the greatest variation among all sampling periods, occurring alongside regular precipitation inputs and declining soil moisture. At the beginning of the study period in April, all three species showed relatively high δ_{xyl} values, with *C. korshinskii* displaying the highest among them. This pattern suggests a winter water storage effect and an early-season reliance on stored water, likely associated with isotopic enrichment driven by transpiration (Sánchez-Costa et al., 2015). Similar patterns were observed in temperate forests of Berlin, Germany (Ring et al., 2024), indicating a broader ecological generality of this phenomenon (Ring et al., 2024). However, from early June to mid-July, precipitation events were infrequent and of low magnitude (mostly <5 mm), accompanied by elevated temperatures. During this period, δ_{xylem} values became more depleted, contrasting with simultaneous enrichment in shallow soil water. This isotopic divergence indicates a shift in water uptake from shallow to deeper soil layers, suggesting that deeper water sources played a critical role in sustaining tree water use under drought conditions (Fig.7). Such preferential uptake of deep soil water under dry conditions has been widely recognized as an adaptive strategy for drought resilience (Kühnhammer et al., 2023), and represents an important ecological mechanism enabling plants in and semi-arid environments to maintain water balance and physiological function.

There was no significant difference of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ between xylem and leaf water between these two species in the mixed arbor-shrub forest ($p>0.05$). This finding is consistent with previous observations in *Pinus tabulaeformis*–*Hippophae rhamnoides* mixed forests (Tang et al., 2019) and *Quercus petraea*–*Pinus sylvestris* stands (Bello et al. 2019), suggesting that long-term adaptation to the same environmental conditions may lead to convergence in plant water isotopic composition. Soil water typically serves as the primary source for plant transpiration, particularly in the loess hilly and gully region where deep groundwater is often inaccessible (Gao et al., 2023). According to the assumption of isotopic consistency, xylem water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values should align with the local soil water line (SWL), regardless of the depth of water uptake. However, in this study, xylem water isotopes of all three species deviated from the SWL (Fig.5). The calculated SW-excess ranged from -10.71‰ to 11.70‰ , with average values of $2.43 \pm 4.28\text{‰}$ for *A. vulgaris*, and $-2.52 \pm 3.53\text{‰}$ for *C. korshinskii*. This isotopic deviation may be related to reduced stem water flow during drought periods. As Kühnhammer et al. (2023) suggested, a decline in stem flow under drought stress may limit water uptake, potentially inducing isotopic fractionation in xylem water. This mechanism could partly explain the observed deviations in δ_{xylem} from the SWL during the drought period.

Water use strategy in the arbor-shrub mixed stands

In the ecohydrological monitoring study, we observed distinct seasonal dynamics in plant water use strategies between two mixed species-*A. vulgaris* and *C. korshinskii*. Both of them were capable of utilizing multiple water sources across different soil depths. In spring, when shallow soil moisture was replenished by snowmelt, a substantial proportion of plant water uptake originated from the 0–20 cm soil layer (Figs 7 ,8). However, during the subsequent drought period, the xylem water isotopes of two species became significantly depleted, indicating a shift in primary water uptake from shallow to deeper soil layers (>20 cm). During the drought period, *C. korshinskii* exhibited a shift in water uptake toward deeper soil layers, while *A. vulgaris* predominantly utilized water from middle soil layers. This vertical partitioning of water resources indicates a complementary water-use strategy between the two species, thereby minimizing direct competition. Correspondingly, the PSI showed a decline under drought conditions, suggesting a reduction in interspecific competition and a shift toward neutral or even facilitative interactions. Such coordination in water-use strategies enhances the stability and resilience of mixed-species systems under water-limited conditions.

Our findings align with those of (Brinkmann et al. 2019; Zhao and Wang 2021; Yang et al., 2015), who similarly observed a progressive shift in plant water uptake depth—from shallow to deeper soil layers—in response to increasing drought severity during the transition from wet to dry seasons. This transition in water sources was closely linked to the decline in soil water content (SWC) (Dai et al. 2023). According to (Zeng et al., 2021), plants generally prefer water uptake pathways that require less energy within the root system, and root activity is strongly dependent on soil moisture thresholds.

Stem sap flow rate is widely recognized as a representative indicator of plant transpiration (Paloschi et al., 2021). Reduced plant sensitivity to environmental stress under dry conditions reflects an adaptive regulation of transpiration aimed at conserving water resources. In our study, the sap flow of *A. vulgaris* began to decline following the onset of summer drought, indicating a suppression of transpiration activity. In contrast, the transpiration rate (Tr) of *C. korshinskii* was less affected; although it no longer increased, it remained relatively stable, suggesting a more balanced water-use

strategy. Under drought conditions, *C. korshinskii* may respond by promoting the growth of fine roots in deeper soil layers with relatively stable water availability (Barbeta et al., 2015), thereby maintaining sap flow and physiological activity during periods of surface moisture depletion. Similarly, previous studies have shown that shrubs can prevent physiological damage during extreme drought by closing stomata to reduce transpiration (Zha et al., 2017). This stomatal regulation serves as a key drought adaptation mechanism for maintaining plant function under prolonged water deficit. This adaptive strategy may explain its greater drought resilience compared to coexisting species.

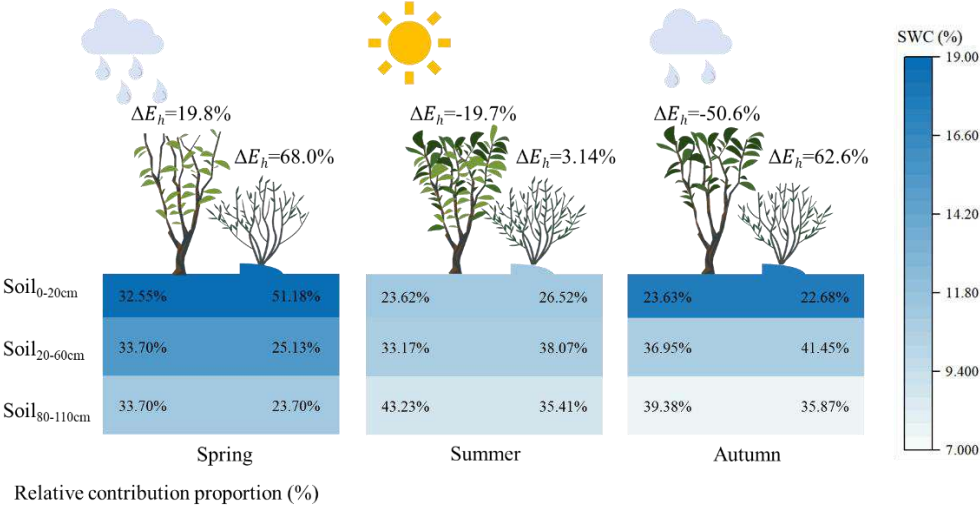


Fig.8 Conceptual graphic of the main ecohydrological fluxes observed in mixed arbor-shrub stands during the spring (April-May), summer drought (July until Mid-August) and under rewetting conditions (Mid-August until September) in 2023. Lighter colors mean drier conditions, darker colors mean moister conditions.

Implications for plantation forest management

A warmer and drier climate has been reported on the Loess Plateau and this trend is predicted to continue in the future according to climate models (Huang et al., 2017). The warming and drying climate together with large-scale revegetation are greatly affecting the limited water resources in this region, leading to runoff reduction (Zhang et al., 2014) and soil desiccation (Gao et al., 2018). These findings suggest that the mixed planting structure of *C. korshinskii* and *A. vulgaris* provides a promising strategy for enhancing ecosystem resilience to drought. Such coordination in water uptake and physiological responses enables the mixed stand to maintain water balance and function under water-limited conditions. Afforestation in the semi-arid Loess Plateau have increasingly recognized the advantages of mixed-species plantations over monocultures. (Che et al. 2023). Moreover, mixed mode also has a directly regulatory effect on plantations growth (Che et al. 2022)

In parallel, the application of level trench plays a crucial role in modifying the soil water environment by increasing infiltration and reducing runoff, particularly on degraded sloped farmland. Integrating these structural measures with biologically diverse vegetation strategies enables a more effective and sustainable approach to ecological restoration. For large-scale Grain-for-Green programs, this dual strategy combining promising water use strategy species selection with hydrological engineering should be prioritized to maximize vegetation establishment, improve

water retention, and promote long-term landscape stability in water-limited regions to reduce drought stress.

Therefore, in the future vegetation restoration construction of the Loess Plateau, the adaptive capacity of different planted species to drought stress and their recovery after extreme drought should be considered simultaneously, and select tree species with complementary traits for mixing planting, and the suitable mixed mode should be determined on the basis of local conditions, to effectively improve the ecosystem quality and stability of plantations in the western Loess Plateau.

Conclusion

In this study, we investigated the response of mixed stands of plantation arbor *A. vulgaris* and shrub *C. korshinskii* in level trench in loess gully area to summer drought stress in the western Loess Plateau. The seasonal variations in transpiration dynamics and water source of mixed *C. korshinskii* and *A. vulgaris* stands were identified by stable isotopes of xylem water, soil water coupled with the MixSIAR model. We found that mixed arbor *A. vulgaris* and shrub *C. korshinskii* stands exists complementary water-use patterns that *C. korshinskii* mainly absorbed deep layer water sources with 43.23% and *A. vulgaris* mainly absorbed middle layer water sources with 38.07% response to summer drought. According to sap flow variations, mixed stands of *A. vulgaris* and *C. korshinskii* indicating higher drought sensitivity and greater resistance and resilience respectively. It's suggested that mixed *C. korshinskii* and *A. vulgaris* has ecological plasticity, as it could explore deeper water sources rapidly and regulate transpiration as the water stress increased. These findings enhance our understanding of plant–soil–atmosphere respond to drought in semi-arid regions and provide valuable implications for the sustainable use of water resources and the maintenance of ecosystem stability in water-limited environments.

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Declarations

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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