Water use characteristics of different pioneer shrubs at different ages in western Chinese Loess Plateau: Evidence from $ $2H offset correction (Postprint)

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Abstract

Caragana korshinskii Kom. and Tamarix ramosissima Ledeb. are pioneer shrubs for water and soil conservation, and for windbreak and sand fixation in arid and semi-arid areas. Understanding the water use characteristics of different pioneer shrubs at different ages is of great importance for their survival when extreme rainfall occurs. In recent years, the stable isotope tracing technique has been used in exploring the water use strategies of plants. However, the widespread $ 2*Hoffsetsofstemwaterfromitspotentialsourcesresultinconflictinginterpretationsofwaterutilizationofpl*
aridareas.Inthisstudy,weusedthreesetsofhydrogenandoxygenstableisotopedata($2H and $ $18O, corrected $ $2H_{c1} based on SW-excess and $ $18O, and corrected $ $2H_{c2} based on −8.1‰ and $ $18O) as inputs for the MixSIAR model to explore the water use characteristics of C. korshinskii and T. ramosissima at different ages and in response to rainfall. The results showed that $ $2H_{c1} and $ $18O have the best performance, and the contribution rate of deep soil water was underestimated because of $ $2H offset. During the dry periods, C. korshinskii and T. ramosissima at different ages both obtained mostly water from deeper soil layers. After rainfall, the proportions of surface (0-10 cm) and shallow (10-40 cm) soil water for C. korshinskii and T. ramosissima at different ages both increased. Nevertheless, there were different response mechanisms of these two plants for rainfall. In addition, C. korshinskii absorbed various potential water sources, while T. ramosissima only used deep water. These flexible water use characteristics of C. korshinskii and T. ramosissima might facilitate the coexistence of plants once extreme rainfall occurs. Thus, reasonable allocation of different plants may be a good vegetation restoration program in western Chinese Loess Plateau.

Full Text

Preamble

Water Use Characteristics of Different Pioneer Shrubs at Different Ages in the Western Chinese Loess Plateau: Evidence from $ $2H Offset Correction

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Abstract: *Caragana korshinskii* Kom. and *Tamarix ramosissima* Ledeb. are pioneer shrubs critical for water and soil conservation, windbreak establishment, and sand fixation in arid and semi-arid regions. Understanding the water use characteristics of different-aged pioneer shrubs is essential for predicting their survival under extreme rainfall events. In recent years, stable isotope tracing techniques have been widely employed to explore plant water use strategies. However, widespread $ 2*Hoffsetsbetweenstemwateranditspotentialsourceshaveledtoconflictinginterpretationsofplantwaterutili*
aridenvironments.Thisstudyutilizedthreesetsofhydrogenandoxygenstableisotopedata($2H and $ $18O, $ $2H_{c1} corrected based on SW-excess and $ $18O, and $ $2H_{c2} corrected based on −8.1‰ and $ $18O) as inputs for the MixSIAR model to investigate the water use characteristics of *C. korshinskii* and *T. ramosissima* at different ages and their responses to rainfall. The results demonstrated that $ $2H_{c1} and $ $18O exhibited the best model performance, and the contribution of deep soil water was underestimated due to the $ $2H offset.

During dry periods, both *C. korshinskii* and *T. ramosissima* at different ages primarily obtained water from deeper soil layers. Following rainfall events, the proportions of surface (0–10 cm) and shallow (10–40 cm) soil water increased for both species across all age classes. Nevertheless, the two species exhibited distinct response mechanisms to rainfall. Additionally, *C. korshinskii* utilized various potential water sources simultaneously, whereas *T. ramosissima* relied predominantly on deep water. These flexible water use characteristics may facilitate plant coexistence during extreme rainfall events, suggesting that strategic allocation of different plant species could represent an effective vegetation restoration approach in the western Chinese Loess Plateau.

Keywords: stable isotope; *Caragana korshinskii*; *Tamarix ramosissima*; water uptake pattern; isotope depletion

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1 Introduction

Water availability is a critical factor governing vegetation growth and ecosystem functioning in arid and semi-arid regions [?, ?]. It constrains vegetation coverage, species richness, biomass production, and biodiversity in ecologically vulnerable areas [?, ?, ?, ?], while also influencing the sustainability of vegetation reestablishment in water-scarce environments [?, ?, ?]. Plants typically satisfy their water requirements from deeper soil layers and groundwater during dry seasons, shifting to shallow soil water uptake during wet periods [?, ?, ?]. Furthermore, species with dimorphic root systems can simultaneously absorb water from both shallow and deep soil horizons [?, ?, ?]. These flexible water use characteristics reflect ecological plasticity that benefits plant growth, reproduction, and competitive ability [?, ?]. Consequently, understanding the water use characteristics of different species is crucial for elucidating interspecific competition and modeling hydrological processes within the soil-plant-atmosphere continuum [?, ?, ?].

Stable isotopes of hydrogen and oxygen have been extensively employed to determine plant water sources [?, ?, ?, ?], based on the assumption that no isotopic fractionation occurs during root water uptake [?]. The isotopic composition of plant xylem water can thus be interpreted as a weighted average of potential water sources and their relative contributions [?]. However, [?] first reported that \$ \$2H values of vacuum-extracted xylem water from mangroves were more depleted than source water, attributing this to hydrogen isotope fractionation during plant water uptake. [?] subsequently documented 3‰-9‰ depletions in \$ \$2H values of xylem water across sixteen xerophytic and semi-xerophytic trees under controlled conditions. Such hydrogen isotope depletion is not an isolated phenomenon but has been observed across diverse taxa, including semi-arid shrubs [?], coniferous and broadleaf forests [?, ?, ?, ?, ?], and tropical rainforests [?, ?]. Failure to account for these \$ \$2H offsets can lead to contradictory interpretations of plant water utilization [?, ?].

Caragana korshinskii Kom., a leguminous shrub with a well-developed root system and strong adaptability, represents a preferred species for soil and water conservation in water-limited regions [?, ?]. *Tamarix ramosissima* Ledeb. is a drought-tolerant, salt-alkali-resistant shrub that plays a vital role in wind prevention and sand fixation on the Chinese Loess Plateau [?]. In this region, deep groundwater is inaccessible to vegetation, forcing plants to rely exclusively on soil moisture replenished by rainfall [?, ?]. Recent climate shifts in northwestern China from warm-drying to warm-wetting conditions [?, ?] will inevitably alter plant water use patterns. Therefore, assessing whether these two pioneer shrubs can adjust their water use characteristics in

response to increasingly variable rainfall patterns is critical for their long-term survival. Previous research indicates that *T. ramosissima* primarily utilizes deep soil water [**?**, **?**], while *C. korshinskii* exhibits pronounced seasonal variation in water source uptake [**?**, **?**]. However, these studies did not account for $ $2H offsets in plant xylem water. [**?**] demonstrated that single-isotope tracers are insufficient for identifying plant water sources when stem water isotopic composition matches multiple potential sources. Consequently, correcting $ *2Hoffsetsisnecessaryforaccuratelyquantifyingrootwateruptake*[**?**]*.Thisstudyinvestigatedthewaterusecha*
C.korshinskii∗and∗T.ramosissima∗atdifferentagesfollowingrainfalleventsontheChineseLoessPlateauusi
(1)*evaluatetheperformanceofthreeisotopedatasets*($2H and $ $18O, $ $2H_{c1} corrected based on SW-excess and $ $18O, and $ $2H_{c2} corrected based on −8.1‰ and $ $18O) as inputs for the MixSIAR model; and (2) investigate the water use characteristics and rainfall responses of *C. korshinskii* and *T. ramosissima* at different ages.

2.1 Study Area

This study was conducted in the western Chinese Loess Plateau near Lanzhou City, Gansu Province (36°07 N, 103°44 E; [Figure 1: see original paper]). The region experiences a mid-temperate continental climate with mean annual precipitation ranging from 270 to 320 mm, unevenly distributed throughout the year and concentrated primarily from June to September. The annual mean air temperature is 10.0°C, with minimum and maximum values of -9.0°C in January and 29.9°C in July, respectively. The terrain slopes from higher elevations in the north to lower elevations in the south, with altitudes ranging from 1560 to 2067 m a.s.l. and slopes generally exceeding 30° [**?**]. The soil is predominantly light sierozem with pH values of 8.0–9.0. Vegetation cover is sparse, consisting mainly of shrubs such as *Tamarix ramosissima*, *Caragana korshinskii*, and *Reaumuria soongorica* (Pall.) Maxim., and herbaceous species including *Agropyron cristatum* (L.) Gaertn. and *Peganum multisectum* (Maxiam.) Bobr. [**?**].

2.2 Experimental Design and Sample Collection

Twig xylem samples from juvenile, intermediate, and adult *C. korshinskii* and *T. ramosissima* were collected from July to October 2020 (Table 1). Sampling occurred on days 1, 3, and 5 following rainfall events on 17–18 July (12.9 mm) and 23 August (15.0 mm), with all collections performed between 08:00 and 10:00 LST. Three replicate samples were obtained from each shrub. Twig xylem segments 3–5 cm long and approximately 0.5 cm in diameter were excised from healthy plants, immediately debarked to retain only xylem tissue, placed in screw-cap glass vials, sealed with parafilm, and stored at -20°C. Concurrently, a 200-cm soil core was excavated adjacent to each sampled shrub, with soil samples collected at depths of 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, and 200 cm (two replicates per individual). Each soil sample was divided: one portion was placed in glass vials, sealed with parafilm, and stored at -20°C for isotopic analysis, while the other was oven-dried at 105°C for

24 h to determine gravimetric water content (SWC, %). Rainfall samples were collected throughout 2020 using a polyethylene tank and funnel apparatus fitted with a ping-pong ball, installed at the meteorological field station of Northwest Normal University' s new campus approximately 2.5 km from the sampling site. Temperature and precipitation data for 2020 were obtained from the nearest Gaolan meteorological station (China Meteorological Data Service Centre).

2.3 Isotopic Analyses

All xylem and soil water samples were extracted using an automatic cryogenic vacuum distillation system (LI-2200, BJJL, Beijing, China) at the Stable Isotope Laboratory, College of Geography and Environmental Science, Northwest Normal University. The vacuum threshold was maintained below 1 Pa/s, with a heating temperature of 105°C and extraction duration of 3 h. A subset of samples was randomly selected, weighed post-extraction, and oven-dried at 105°C for 24 h to verify extraction efficiency exceeded 98% [?]. The isotopic compositions of extracted soil water, xylem water, and rainfall samples were measured using isotope ratio infrared spectroscopy (IRIS; T-LWIA-45-EP, ABB-Los Gatos Research, CA, USA) with analytical precisions of $±$1‰ for $ $2H and $±$0.3‰ for $ $18O. Isotopic compositions are expressed in per mil (‰) relative to Vienna Standard Mean Ocean Water (VSMOW) according to:

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000‰$$

where R_{sample} and R_{standard} represent the $^{2}\text{H}/^{1}\text{H}$ or $^{18}\text{O}/^{16}\text{O}$ ratios of the sample and VSMOW standard, respectively. Organic contaminants (e.g., methanol, ethanol, and other biogenic volatile compounds) in cryogenically extracted xylem and soil water can affect IRIS measurements [?]. Therefore, spectral analysis software from Los Gatos Research (ABB-Los Gatos Research, CA, USA) was employed to correct isotope data based on measured absorption spectra indices, thereby eliminating organic contamination effects [?, ?].

2.4 Data Analysis

[?] proposed line-conditioned excess (lc-excess) to evaluate $ $2H offsets between soil water (or river/groundwater) and precipitation:

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

where a and b are the slope and intercept of the local meteoric water line (LMWL), respectively. The lc-excess quantifies non-equilibrium dynamic fractionation caused by evaporation [?], with precipitation averaging 0‰ and evaporatively enriched water bodies (e.g., soil water, river water) typically exhibiting values less than 0‰. Since plant water originates from soil rather than directly from precipitation, [?] revised this concept and proposed SW-excess to describe deviations of plant xylem water from the soil water line (SWL):

$$\text{SW-excess} = \delta^2\text{H} - a_s\delta^{18}\text{O} - b_s$$

where a_s and b_s represent the slope and intercept of the SWL at each sampling point during a given period. Positive SW-excess indicates xylem samples are more enriched in $ $2H than the SWL, while negative values indicate depletion.

The corrected hydrogen isotope composition of xylem water ($ $2H_{c1}) is calculated as:

$$\delta^2\text{H}_{c1} = \delta^2\text{H} - \text{SW-excess}$$

Additionally, [?] identified cryogenic extraction bias in plant xylem water, attributing hydrogen isotope depletion to dynamic exchange between organic-bound deuterium and water. They proposed a correction method based on stem relative water content:

$$\delta^2\text{H}_{c2} = \delta^2\text{H} - \varepsilon \pm S$$

where $\delta^2\text{H}_{c2}$ is the corrected stem water hydrogen isotope value, ε represents the deuterium offset between cryogenically extracted stem water and true xylem water (expressed as a regression function of stem relative water content), and S denotes measurement uncertainty [?]:

$$S = \sqrt{0.5^2(\text{analytical error}^2_{\delta^2\text{H}} + \text{analytical error}^2_{\delta^{18}\text{O}})}$$

Since stem relative water content was not measured, we treated ε as a constant value of -8.1‰, corresponding to the species-averaged offset between $ *2H{stem_CVD}$ (hydrogen isotope ratio of cryogenically extracted stem water) and $ 2H*{xylem}$ (hydrogen isotope ratio of plant source water) reported by [?].

Various methods exist for tracing plant water sources using stable isotopes, including graphical inference, two- or three-source linear mixing models, the multiple linear mixing model (IsoSource), and Bayesian mixing models (MixSIR, SIAR, and MixSIAR). Among these, MixSIAR accounts for uncertainty in root water uptake while providing optimal solutions rather than a range of feasible outcomes [?, ?, ?, ?]. Therefore, we employed the Bayesian mixing model MixSIAR to estimate contributions from different potential water sources. Assuming no isotopic fractionation during root water uptake [?, ?], the discrimination factor was set to 0. The Markov Chain Monte Carlo (MCMC) run length was configured as 'long' (chain length = 300,000; burn-in = 200,000; thin =

100; chains = 3) to ensure model convergence, which was verified using Gelman-Rubin and Geweke diagnostics. Model outputs are presented as mean values.

To assess the impact of $ $2H offset on quantifying root water uptake, we used three isotope datasets as MixSIAR inputs: (1) $ $2H and $ $18O, (2) $ $2H_{c1} and $ $18O, and (3) $ $2H_{c2} and $ $18O. Model performance was evaluated using Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and root mean square error (RMSE), with the dataset yielding the lowest values considered optimal:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(p_i - o_i)^2}$$

where n is the number of validation samples, and p_i and o_i are predicted and observed xylem water isotope values, respectively.

One-way ANOVA with least significant difference (LSD) tests ($P < 0.05$) were used to examine isotopic differences among water bodies; $P \geq 0.05$ indicated no significant difference. Pearson correlation analysis assessed relationships between potential soil water source contributions and environmental factors (rainfall, vapor pressure deficit, and soil water content). The sampling profile was divided into four potential soil water sources based on variability in $ $2H, $ $18O, and SWC: surface (0-10 cm), shallow (10-40 cm), middle (40-100 cm), and deep (100-200 cm) soil water.

3.1 Soil Water Content

Vertical distributions of soil water content for *C. korshinskii* and *T. ramosissima* at different ages are illustrated in Figure 2 [Figure 2: see original paper]. The 0-40 cm soil layers of both species showed relatively higher water contents, with more pronounced temporal variation after rainfall events compared to the 40-100 cm layer, indicating greater susceptibility to rainfall infiltration and evaporation in surface horizons. The 40-100 cm layer exhibited smaller seasonal differences with gentle fluctuations, while soil water content below 100 cm remained lowest and showed minimal variation. Profile-wide soil water content was lowest before rainfall (9 July and 16 August), increased rapidly one day after rainfall (19 July and 24 August), and gradually decreased thereafter.

3.2 Isotopic Composition of Different Water Bodies

Precipitation $ $18O values ranged from -18.06‰ to 1.58‰, with an annual precipitation-weighted mean of -8.21‰. $ $2Hvaluesrangedfrom–126.82‰to–28.73‰,withanannualweighted$ = -12.16‰, $ $18O = –2.91‰),$whileminimaoccurredinJanuary$($2H = -96.41‰, $ $18O = -13.72‰) (Fig. S1). The LMWL was defined as $ $2H = 6.95(\pm0.26)\ 18O + 3.25(\pm2.31)$(R^2 = 0.95$, $P < 0.01$), with slope and intercept lower than the Global Meteoric Water Line (GMWL) [**?**], reflecting intense evaporation characteristic of arid and semi-arid climates (Fig. 3 [Figure 3: see original paper]).

Hydrogen and oxygen isotopes in soil water showed similar vertical and temporal patterns; therefore, $ $18O was used to characterize soil water isotopic composition (Figs. S2 and S3). Soil water $ $18O varied with depth and species (Fig. S3; Table S1), with the 0–40 cm layer showing greater temporal variability than the 40–100 cm layer, which stabilized with increasing depth. Significant differences in soil water $ 18*Oexistedbetweenthetwoshrubspeciesatdifferentages*(P < 0.05$). Mean soil water $ 18*Ovalueswere*–4.69‰(±3.39‰)*forjuvenile* ∗ *C.korshinskii*∗,–5.27‰(±3.14‰)*forintermediate*,–7.01‰(±3.47‰)*foradult*,–4.27‰(±3.61‰)*forjuvenil*
T.ramosissima∗,–1.06‰(±4.38‰)*forintermediate*, *and*–0.38‰(±$3.80‰) for adult. Prior to rainfall (9 July and 16 August), soil water isotopes were most enriched, decreased dramatically one day after rainfall (19 July and 24 August), and gradually increased thereafter due to evaporative enrichment [**?**] (Fig. S3). Relationships between $ $2H and $ $18O in soil water for both species at different ages are shown in Figure 3. SWL slopes increased with age for *C. korshinskii* but decreased for *T. ramosissima.* All SWLs plotted to the lower right of the LMWL and intersected it, indicating soil water originated from precipitation but was subjected to varying degrees of evaporation.

Significant interspecific differences in xylem water isotope values ($P < 0.05$) suggested distinct water use characteristics between *C. korshinskii* and *T. ramosissima.* Age-related differences in xylem water isotopes were significant for *C. korshinskii* ($P < 0.05$) but not for *T. ramosissima* ($P \geq 0.05$). Isotopic compositions of both species at different ages closely matched soil water values, confirming that plants primarily obtained water from soil horizons (Fig. 3). No significant variation in xylem water isotopic composition occurred immediately after the 17-18 July and 23 August rainfall events (Table S1), likely because rainfall isotopic signals required time to translocate to plant xylem.

3.3 $ $2H Offset of Xylem Water

The $ $2H offset between xylem water and SWL was quantified using SW-excess. Mean SW-excess values were 2.43‰, 0.42‰, and 3.48‰ for juvenile, intermediate, and adult *C. korshinskii*, respectively, and -1.03‰, 3.16‰, and 1.85‰ for juvenile, intermediate, and adult *T. ramosissima*, respectively (Fig. 4 [Figure 4: see original paper]). These findings differ from previous studies, with both positive and negative SW-excess values potentially related to rainfall event characteristics. Mean SW-excess after the 17-18 July rainfall (SW-excess > 0) was greater than after the 23 August rainfall (SW-excess < 0), attributable to lower SWL slopes following the July event (except for intermediate *T. ramosissima*) (Fig. S4). This difference stemmed from contrasting rainfall isotopic compositions: $ $2H = -33.26‰, $ $18O = -5.64‰ on 17-18 July versus $ $2H = -68.76‰, $ $18O = -11.43‰ on 23 August. Compared to uncorrected $ $2H, both corrected $ $2H_{c1} and $ $2H_{c2} values fell within the range of potential water sources (Fig. 3), validating the correction methods.

3.4 Water Source Apportionment by Three Input Datasets

When isotopic compositions of potential water sources are similar, single-isotope analysis becomes insufficient for distinguishing contribution rates among different sources. Therefore, we employed three isotope datasets as MixSIAR inputs: (1) $ $2H and $ $18O, (2) $ $2H_{c1} and $ $18O, and (3) $ $2H_{c2} and $ $18O, yielding different results (Figs. 5 and 6). AIC, BIC, and RMSE values indicated that $ $2H_{c1} and $ $18O provided the best performance for identifying water use characteristics (Table 2). In contrast, $ $2H_{c2} and $ $18O did not outperform uncorrected $ $2H and $ $18O because we used a constant value of -8.1‰ rather than measured stem water content. This suggests that -8.1‰ cannot serve as a universal correction factor. Moreover, hydrogen isotope offset led to underestimation of deep soil water contributions (Table S2). Consequently, $ $2H_{c1} and $ $18O datasets were used for analyzing root water uptake patterns of pioneer shrubs at different ages.

Table 2 Performance of water source contribution using three input datasets to the MixSIAR model

Input dataset	AIC	BIC	RMSE
$ $2H and $ $18O	[value]	[value]	[value]
$ $2H_{c1} and $ $18O	[value]	[value]	[value]
$ $2H_{c2} and $ $18O	[value]	[value]	[value]

Note: AIC, Akaike information criterion; BIC, Bayesian information criterion; RMSE, root mean square error.

Relative proportions of soil water sources for *C. korshinskii* at different ages after rainfall, calculated using the MixSIAR model with the three input datasets, are shown in Figure 5 [Figure 5: see original paper]. On 9 July, intermediate and adult *C. korshinskii* obtained most water from 100-200 cm soil layers, coinciding with higher water content at these depths (no juvenile samples were collected). Following the 17-18 July rainfall, juvenile *C. korshinskii* absorbed water from all four soil layers at 1 day post-rainfall, then shifted to primarily using 10-40 and 40-100 cm layers at 3 and 5 days. On 16 August, juvenile *C. korshinskii* favored 10-40 and 40-100 cm layers, shifting to 0-10 cm water at 3 and 5 days after the 23 August rainfall, with 0-10 cm contributions increasing over time. During the late growing season (September-October), juvenile *C. korshinskii* mainly utilized 0-10 and 10-40 cm soil water, with proportions gradually increasing. Intermediate *C. korshinskii* extracted 0-40 cm water at 1 day after the July rainfall, with contributions from this zone decreasing at 3 and 5 days. On 16 August, intermediate plants used mostly 0-10 cm water, shifted to 10-40 cm at 1 day after the August rainfall, then returned to 0-10 cm at 3 and 5 days. During late season, intermediate plants primarily used 0-10 and 10-40 cm water, with proportions gradually decreasing. Adult *C. korshinskii* used 40-100 cm water at 1 day after the July rainfall, gradually increasing 0-40 cm contributions by 5 days. On 16 August, adults utilized mostly 0-10 cm water, then used both 0-10 cm (50.7% ± 3.7%) and 100-200 cm (43.1% ± 2.1%) at 1 day after the August rainfall, with 0-40 cm contributions steadily increasing thereafter. During late season, adult plants mainly absorbed 0-10 and 10-40 cm water, with proportional contributions gradually increasing.

Figure 6 [Figure 6: see original paper] shows relative water source proportions for *T. ramosissima* at different ages after rainfall, calculated using the three input datasets. On 9 July, juvenile and intermediate *T. ramosissima* primarily used 100-200 cm water, while adults absorbed water from both 40-100 and 100-200 cm layers. Following the 12.9 mm rainfall on 17-18 July, juvenile plants used 0-10 and 100-200 cm water at 1 day post-rainfall, shifting to 100-200 cm by 3 and 5 days. Intermediate plants showed >70% contribution from 0-10 cm at 1 day, with 10-40 cm contributions increasing at 3 and 5 days. Adult plants exhibited >70% 0-10 cm contributions at 1 day, increasing dramatically to 98% at 3 days, then shifting from 0-10 cm at 3 days back to 100-200 cm at 5 days. On 16 August, juvenile and adult plants extracted primarily 40-100 cm water, while intermediates used 10-40 cm. After the 15.0 mm rainfall on 23 August, juvenile plants showed approximately equal contributions from all layers at 1 day, then used 90% 0-10 cm water at 3 days, with 0-10 and 10-40 cm contributions remaining high at 5 days. Intermediate plants used 0-10 and 10-40 cm water at 1 day, switched to 10-40 and 100-200 cm at 3 days, and used 70% 10-40 cm at 5 days. Adult plants absorbed 0-10 and 10-40 cm water at 1 day, shifted to 0-10 cm at 3 days, and used 70% 40-100 cm at 5 days. In September, 100-200 cm water was the primary source for juvenile and adult plants, while intermediates used 40-100 cm. In October, juvenile, intermediate, and adult plants showed highest proportions from 40-100 and 100-200 cm, 100-200 cm (90%), and 40-100 cm (90%), respectively.

4.1 Variation in Stable Isotopes of Soil Water after Rainfall

On the Loess Plateau, deep groundwater is inaccessible to vegetation, forcing plants to rely exclusively on soil moisture derived from rainfall [?]. This study revealed more dramatic temporal variation in soil water content and isotopic composition in the 0-40 cm layer compared to deeper horizons after rainfall (Figs. 2, S2, and S3), indicating greater susceptibility to rainfall infiltration and evaporation [?]. This aligns with [?], who documented fading fractionation effects with depth: stable isotopes in upper soil water varied substantially upon rainfall infiltration, with effects diminishing at depth. Pre-rainfall soil water isotopes (9 July and 16 August) were most enriched, decreasing dramatically one day post-rainfall (19 July and 24 August) due to infiltration of isotopically negative rainfall, then gradually increasing at 3 and 5 days due to evaporative enrichment [?].

4.2 Water Uptake Patterns of *C. korshinskii* and *T. ramosissima* at Different Ages

Caragana korshinskii increased its reliance on deeper (100–200 cm) soil water with age. Juvenile and intermediate plants primarily used 0–10 and 10–40 cm layers, while adults mainly absorbed water from 40–100 and 100–200 cm layers. During dry periods (9 July), intermediate and adult plants obtained most water from deeper (100–200 cm) layers (no juvenile data available). Following rainfall, *C. korshinskii* at different ages shifted water sources among soil layers (Figs. 5 and 6), with age-specific responses to rainfall (Tables S3 and S4). When antecedent soil water was deficient, juvenile plants exhibited more homogeneous water uptake across soil horizons after rainfall (post-17–18 July). Conversely, when antecedent soil water was sufficient, juvenile plants showed greater sensitivity to rainfall, shifting main water sources from 100–200 cm to 0–10 cm after the 23 August rainfall. This pattern mirrors [?], who found that *Haloxylon ammodendron* water use strategies were more sensitive to rainfall in spring with abundant surface soil water than in dry summer. Intermediate *C. korshinskii* responded immediately to rainfall, tending to use 0–40 cm water after both rainfall events. Adult plants showed delayed responses, with 0–40 cm contributions gradually increasing at 1, 3, and 5 days post-rainfall.

Tamarix ramosissima is a deep-rooted species whose surface roots absorb rainfall-supplied moisture while deep roots access water from winter/spring precipitation or groundwater during drought [?, ?, ?]. Surface roots may become dormant under dry conditions and reactivate after rainfall, allowing plants to rely exclusively on deep roots for water uptake when rainfall is scarce and shallow soil water is limited [?]. When rainfall reaches a threshold that increases shallow soil moisture, surface roots resume function, reducing energy consumption [?, ?, ?]. During dry periods (9 July), juvenile and intermediate *T. ramosissima* used deeper (100–200 cm) water, while adults used middle (40–100 cm) and deep (100–200 cm) layers. After rainfall, plants of all ages tended to absorb surface (0–10 cm) and shallow (10–40 cm) water supplied by rainfall, consistent with [?] in the Gobi region of Dunhuang, China. Age-specific responses to rainfall varied (Tables S3 and S4). Juvenile plants were sensitive to rainfall when antecedent soil water was sufficient, while intermediate and adult plants responded immediately. Intermediate plants tended to use 0–40 cm water after both rainfall events, with proportions gradually decreasing. Adult plants tended to absorb 100–200 and 40–100 cm water at 5 days after the July and August rainfall events, respectively.

Contribution rates of potential water sources for both species at different ages correlated with environmental data (rainfall, vapor pressure deficit, and soil water content) (Table S5). For *C. korshinskii*, discrepancies in water source contributions primarily related to soil water content, whereas for *T. ramosissima*, differences correlated with cumulative rainfall 7 days before sampling, average vapor pressure deficit 7 days before sampling, and soil water content.

4.3 Implications

Climate transition from warm-drying to warm-wetting and increasing extreme precipitation frequency on the Loess Plateau will compel plants to alter water use patterns [**?**]. During dry periods, intermediate and adult *C. korshinskii* and juvenile/intermediate *T. ramosissima* relied primarily on deeper (100-200 cm) soil water, while adult *T. ramosissima* used middle (40-100 cm) and deeper (100-200 cm) layers. After rainfall, proportions of surface (0-10 cm) and shallow (10-40 cm) water increased for both species across age classes, though response mechanisms differed. *Caragana korshinskii* utilized multiple potential water sources simultaneously, whereas *T. ramosissima* relied predominantly on deep water. These divergent strategies enable complementary resource utilization and promote species coexistence, providing valuable guidance for species selection and management in Loess Plateau vegetation restoration. Restoration efforts should combine plants with different water use characteristics to create optimal plant configurations that compensate for interspecific water use limitations. This study evaluated three datasets: $ $2H and $ $18O, $ $2H_{c1} corrected using SW-excess, and $ $2H_{c2} corrected using -8.1‰. The $ $2H_{c2} and $ $18O performance was suboptimal because we used a constant value rather than measured stem water content; future studies using measured stem water content may achieve better correction. Additionally, fine roots determine water uptake, necessitating long-term research on root biomass, activity-related characteristic changes, and temporal stable isotope dynamics.

5 Conclusions

This study used three hydrogen and oxygen stable isotope datasets as MixSIAR inputs to investigate water use strategies and rainfall responses of *C. korshinskii* and *T. ramosissima* at different ages in the western Chinese Loess Plateau. Model performance evaluation revealed that $ $2H_{c1} and $ $18O provided the best results, while $ $2H offset caused underestimation of deep soil water contributions. During dry periods, deeper (100-200 cm) soil water was the primary source for intermediate and adult *C. korshinskii*, juvenile and intermediate *T. ramosissima*, while middle (40-100 cm) and deeper (100-200 cm) water sustained adult *T. ramosissima* (no juvenile *C. korshinskii* data). After rainfall, proportions of surface (0-10 cm) and shallow (10-40 cm) water increased for both species across all ages, though responses varied. These flexible water use characteristics may facilitate plant coexistence during extreme rainfall events, providing important insights for vegetation restoration on the Chinese Loess Plateau.

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Appendix

Table S1 Isotopic composition of different water bodies

Sample	$ $2H (‰)	$ $18O (‰)
Precipitation	-49.91*	-8.21*
Juvenile *C. korshinskii*	[range]	[range]
Intermediate *C. korshinskii*	[range]	[range]
Adult *C. korshinskii*	[range]	[range]
Juvenile *T. ramosissima*	[range]	[range]
Intermediate *T. ramosissima*	[range]	[range]
Adult *T. ramosissima*	[range]	[range]

Note: represents annual weighted mean precipitation.*

Table S2 Contributions of three data input modes to the MixSIAR model

Input data mode	0–10 cm	10–40 cm	40–100 cm	100–200 cm
$ $2H and $ $18O	[value]	[value]	[value]	[value]
$ $2H_{c1} and $ $18O	[value]	[value]	[value]	[value]
$ $2H_{c2} and $ $18O	[value]	[value]	[value]	[value]

Note: SD, standard deviation.

Table S3 Contribution of 0–40 cm soil water for *C. korshinskii* at different ages after rainfall

Plant	Date	Contribution (%) \$ \$2H and \$ \$18O	Contribution (%) \$ \$2H_{c1} and \$ \$18O	Contribution (%) \$ \$2H_{c2} and \$ \$18O
Juvenile *C. korshinskii*	19 Jul (1 d after rainfall)	[value]	[value]	[value]
Intermediate *C. korshinskii*	21 Jul (3 d after rainfall)	[value]	[value]	[value]
Adult *C. korshinskii*	24 Aug (1 d after rainfall)	[value]	[value]	[value]
Juvenile *C. korshinskii*	26 Aug (3 d after rainfall)	[value]	[value]	[value]
Intermediate *C. korshinskii*	24 Aug (1 d after rainfall)	[value]	[value]	[value]

Plant	Date	Contribution (%) \$ \$2H and \$ \$18O	Contribution (%) \$ \$2H_{c1} and \$ \$18O	Contribution (%) \$ \$2H_{c2} and \$ \$18O
Adult *C. korshinskii*	28 Aug (5 d after rainfall)	[value]	[value]	[value]

Table S4 Contribution of 0–40 cm soil water for *T. ramosissima* at different ages after rainfall

Plant	Date	Contribution (%) \$ \$2H and \$ \$18O	Contribution (%) \$ \$2H_{c1} and \$ \$18O	Contribution (%) \$ \$2H_{c2} and \$ \$18O
Juvenile *T. ramosissima*	19 Jul (1 d after rainfall)	[value]	[value]	[value]
Intermediate *T. ramosissima*	21 Jul (3 d after rainfall)	[value]	[value]	[value]
Adult *T. ramosissima*	24 Aug (1 d after rainfall)	[value]	[value]	[value]

Plant	Date	Contribution (%) \$ \$2H and \$ \$18O	Contribution (%) \$ \$2H_{c1} and \$ \$18O	Contribution (%) \$ \$2H_{c2} and \$ \$18O
Juvenile *T. ramosissima*	24 Aug (1 d after rainfall)	[value]	[value]	[value]
Intermediate *T. ramosissima*	24 Aug (1 d after rainfall)	[value]	[value]	[value]
Adult *T. ramosissima*	28 Aug (5 d after rainfall)	[value]	[value]	[value]

Table S5 Correlation between contribution calculated by \$ \$2H_{c2} and \$ \$18O input into the MixSIAR model and cumulative rainfall amount 7 days before sampling

Plant	Depth	Rainfall (7 d amount)	VPD (7 d mean)	SWC
Juvenile *C. korshinskii*	0–10 cm	-0.688*	-0.876**	-0.712*
	10–40 cm	-0.686*	0.659*	0.685*
	40–100 cm	0.669*	0.718*	
Intermediate *C. korshinskii*	0–10 cm	0.799*	0.826**	0.795**
Adult *C. korshinskii*	0–10 cm	-0.636*	0.688*	
Juvenile *T. ramosissima*	0–10 cm	-0.688*	-0.876**	-0.712*
Intermediate *T. ramosissima*	0–10 cm	0.799*	0.826**	0.795**
Adult *T. ramosissima*	0–10 cm	-0.636*	0.688*	

Note: VPD, vapour pressure deficit; SWC, soil water content; , $P < 0.05$; **, P

< 0.01.*

Fig. S1 Variation of monthly precipitation-weighted $ $2H and $ $18O. No precipitation data for February, March, April, and November.

Fig. S2 Variation of soil water $ $2H in juvenile *C. korshinskii* (a1–a3), intermediate *C. korshinskii* (b1–b3), adult *C. korshinskii* (c1–c3), juvenile *T. ramosissima* (d1–d3), intermediate *T. ramosissima* (e1–e3), and adult *T. ramosissima* (f1–f3).

Fig. S3 Variation of soil water $ $18O in juvenile *C. korshinskii* (a1–a3), intermediate *C. korshinskii* (b1–b3), adult *C. korshinskii* (c1–c3), juvenile *T. ramosissima* (d1–d3), intermediate *T. ramosissima* (e1–e3), and adult *T. ramosissima* (f1–f3).

Fig. S4 Linear regression relationship between $ $2H and $ $18O in soil water after rainfall on 17–18 July and 23 August in juvenile, intermediate, and adult *C. korshinskii* (a–c), and juvenile, intermediate, and adult *T. ramosissima* (d–f). LMWL is the local meteoric water line.