

Water use characteristics of the common tree species in different plantation types in the Loess Plateau of China

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Abstract

Knowledge concerning the water use characteristics of revegetated species has profound implications for understanding soil–plant interaction mechanisms and guiding ecological restoration strategies in water-limited ecosystems. Although afforestation is an important way to improve ecosystem functions and services in degraded ecosystems, there is limited understanding about the water use characteristics of dominant species within and between different types of plantations. We investigated plant water use characteristics in three representative types of plantations on the Chinese Loess Plateau: mixed plantation consisting of three deciduous tree species *Robinia pseudoacacia*, *Armeniaca sibirica* and *Ailanthus altissima* (Mspa), pure *R. pseudoacacia* plantation (Pp) and pure *A. sibirica* plantation (Ps). We measured the leaf $\delta^{13}\text{C}$ of the dominant species within each plantation type and the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of xylem and soil water within 400 cm of the soil surface. The results showed that three main species in the mixed plantation exhibited significant difference ($p < 0.05$) in proportional contributions of water sources, suggesting that the plants had water source segregation. *A. sibirica* in the mixed plantation utilized more proportional shallow soil water than that in the pure plantation and correspondingly lessened deep soil water depletion. However, no significant difference was found in the water uptake proportions of *R. pseudoacacia* between the different plantation types. The leaf $\delta^{13}\text{C}$ values of the plant species in the mixed plantation were significantly higher than those in the pure plantations. The leaf $\delta^{13}\text{C}$ values of *R. pseudoacacia* under different plantation were positively associated with SWCs, but this relationship was not observed in *A. sibirica*. These results indicate

that plantation type affected plant water use characteristics with species-specific responses to plantation type and different water source competition effects between interspecific versus intraspecific competition.

Keywords: stable isotopes; plant water use; water use efficiency; mixed plantation; Loess Plateau;

1. Introduction

Drylands cover approximately 40% of the earth's terrestrial surface, and more than two billion people live in dryland regions (Wang et al., 2012a). Approximately 15% of drylands experience different forms of severe land degradation (Reynolds et al., 2007) with negative impacts on biological diversity and peoples' livelihoods due to human activities and climate change. Ecological restoration, including natural regeneration and active restoration (e.g., revegetation), is widely used to restore ecosystem functions, enhance biodiversity and halt land degradation (Birch et al., 2010; Menz et al., 2013). The Chinese Loess Plateau with an area of 640,000 km², which has the largest and deepest loess deposit in the world, is threatened by severe water shortage. The average annual rainfall (MAP) in this region is approximately 400 mm ranging from 200 to 700 mm but annual potential evaporation is consistently greater than 1,000 mm (Fu et al., 2017). The area with MAP from 550 to 700 mm was expected to be suitable for plantation in Loess Plateau (Zhang et al., 2008). The Loess Plateau has achieved successful ecological restoration with various plantation types because of the

implementation of the Grain-for-Green Project, and forest coverage increased to 21.7% (Fu et al., 2017).

Different plantation types with diverse plant species have been adopted for revegetation in dryland ecosystems (Meng et al., 2008), such as mixed plantations and monocultures. The spatial and temporal heterogeneity of resources and environmental conditions in arid and semiarid ecosystems induce ecological niche differentiation which can facilitate species coexistence (Chesson et al., 2004). The plants in water-limited ecosystems developed multiple morphological and physiological traits as acclimation responses to water source variability (e.g., extreme drought and precipitation pulses) (Antunes et al., 2018; Gao et al., 2018), such as root functional traits (Fort et al., 2017), stomatal adjustment and leaf water potential (Altieri et al., 2015; Quero et al., 2011). Plants adopt different strategies to promote the coexistence of different plant species in various ecosystems (Silvertown et al., 2015; Swaffer et al., 2014). One possible interpretation for coexistence in plant communities is hydrological niche segregation, which was defined as spatial or temporal partitioning of fine-scale soil water resources. This partitioning can be achieved via different phenologies, rooting depths and partitioning of supplementary opportunities over time caused by species-specific (Silvertown et al., 2015). Another non-mutually exclusive explanation for plant coexistence is eco-hydrologic separation hypothesis, which states that water sources are separated into two components: tightly bound water that is utilized by plants and mobile water that is unrelated to plant water uptake (Brooks et al., 2009; Evaristo et al., 2015). Thus, understanding the water use strategies of plants for revegetation is

critical to provide knowledge related to soil–plant interactions and ecological restoration management in water-limited ecosystems (Grossiord et al., 2019; Sprenger et al., 2016).

Water source sustainability is a critical concern in revegetation strategies, especially in water-limited regions. Temporal-spatial variations in the water sources used by plants and water use efficiency are the key characteristics of plant water use. Stable hydrogen and oxygen isotope techniques have been applied to determine water use sources (Antunes et al., 2018; Wang et al., 2019a; Grossiord et al., 2019). The $\delta^{13}\text{C}$ in plant leaves have a positive relationship with intrinsic water use efficiency (WUE_i) for C_3 photosynthesis plants and is an indicator of leaf-level WUE_i (Farquhar et al., 1989; Saugier et al., 2012). Stable isotope analysis is a powerful and effective approach for identifying water use characteristics and has been successfully applied in various regions (Ehleringer and Dawson, 1992; Moreno-Gutierrez et al., 2012; Yang et al., 2015) because there is no isotopic discrimination during plant root water uptake (Dawson et al., 2002) except for a few halophytic and xerophyte plants (Ellsworth and Williams, 2007; Lin and Sternber, 1993). Previous studies have used isotope techniques to explore water use strategies for coexisting plants in various ecosystems (Moreno-Gutierrez et al., 2012; Yang et al., 2015; Zhang et al., 2017). For example, Moreno-Gutierrez *et al.* (2012) investigated the water use patterns among coexisting plant species in a Mediterranean ecosystem. Zhang *et al.* (2017) explored differences in the water use strategies of two coexisting shrubs in desert ecosystems. However, the majority of current studies on the water use strategies of coexisting plants have been conducted in

natural ecosystems. Few studies have focused on the water use characteristics of planted forests, especially on the Loess Plateau, which is characterized by large-scale afforestation by anthropogenic activities. In addition, the groundwater on the Loess Plateau is deep, and the soil layer above the groundwater is very thick compared with those in other dryland regions. The plants had difficulty deriving the groundwater and soil water from different layers are the main potential resources for plant water uptake in this region (Wang et al., 2017). However, the proportional contributions of soil water for planted trees remain poorly understood in this region.

Although large vegetation changes have occurred on the Loess Plateau in the past decades, the persistence of revegetation is often questioned in terms of water source (Chen et al., 2015; Gao et al., 2011). For example, soil desiccation in the deep soil layer formed due to the unreasonable introduction of exotic plant species and high-density planting (Wang et al., 2011). Recent studies have investigated the seasonal water uptake patterns of natural and plantation species in revegetated ecosystems of the semiarid Loess Plateau (Lü et al., 2017; Wang et al., 2017). Moreover, the responses of exotic and native shrub species to natural extreme drought were also explored in this region (Wang et al., 2019a). However, in these studies, plant water use characteristics were examined in monoculture plantations. There is limited understanding about the water use strategies of mixed plantation species, particularly the difference between pure and mixed plantation species (Tang et al., 2018).

To fill these knowledge gaps, we applied isotope techniques ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) to determine the water use characteristics of revegetated species in mixed and pure

plantations with monthly sampling during the growing season on the semiarid Loess Plateau. The primary objectives of this study were to (i) investigate the seasonal water use characteristics of species in mixed plantations and (ii) determine the difference in water use characteristics of species between mixed and pure plantations and (iii) explore the consequences of interspecific (in pure plantations) versus intraspecific competition (in mixed plantations) for soil water and plant water use. The hypotheses are that the plant species in the mixed and pure plantations would exhibit different water use characteristics and that coexisting plant species would show hydrological niche segregation.

2. Materials and methods

2.1 Field site

This study was conducted in the Yangjuangou catchment in Shaanxi Province of China (36°42'45" N, 109°31'45") (Fig. 1). This catchment is a loess gully and hilly region in the central region of the Loess Plateau. The total area of the catchment is 2.02 km², with an average elevation of 1,295 m. The average annual (1961–2016) precipitation and air temperature are 537 mm and 10°C, respectively (Wang et al., 2017). The soil is classified as typical loess with a fine silt texture. The main land-use types are woodlands, shrubs, grasslands, orchards and farmlands. The major forest, shrub and orchard plantations are located on the steep slopes and grassland has been naturally resorted since the Grain-for-Green Project was implemented in 1999. The major forest species are *Robinia pseudoacacia*, *Armeniaca sibirica*, *Ailanthus altissima*, and

Juglans regia. The main shrub species are *Hippophae rhamnoides*, *Spiraea trilobata*, *Vitex negundo*, and *Sophora viciifolia*. The grassland is dominated by *Stipa bungeana* and *Artemisia gmelinii*.

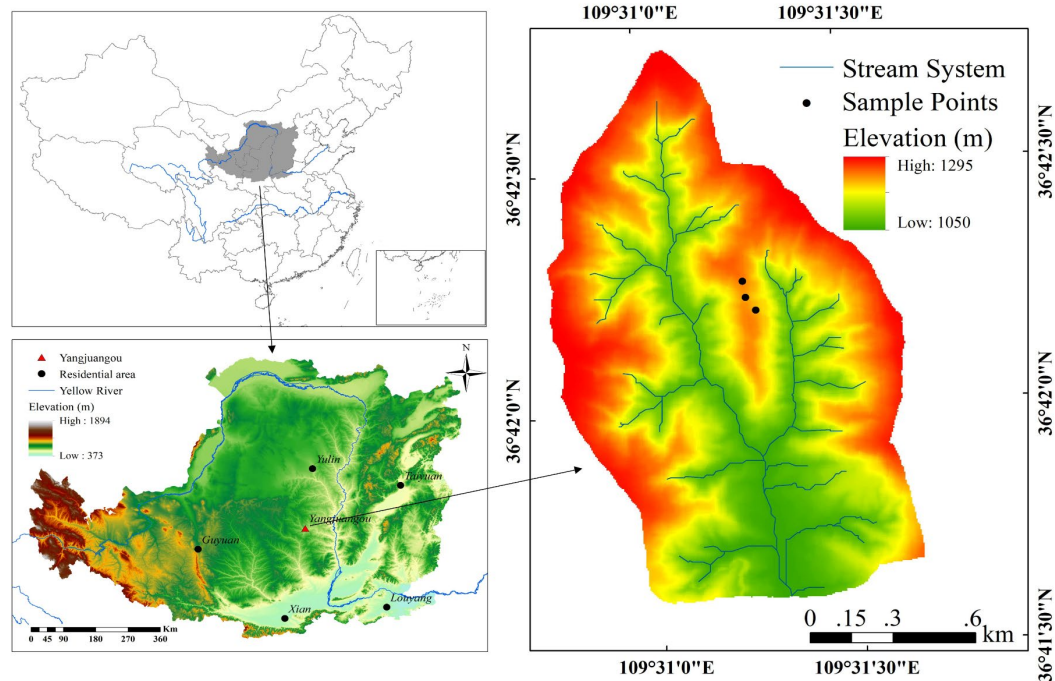


Fig. 1. The geographical location of the study site.

2.2 Experimental design and sample collection

We selected three types of planted forests in the Yangjuangou catchment. The mixed forest plantation consisted of *R. pseudoacacia*, *A. sibirica* and *A. altissima* (Mspa), and the pure forest plantations were *R. pseudoacacia* (Pp) and *A. sibirica* (Ps). Three plots (10 m × 10 m) were established for each type of plantation. The spatial distance among the tree types of plantation were less than 1 km and had similar micro-environment, including soil condition, micro-geomorphology. The spatial distribution of trees in each plantation was relatively uniform. Each plot had similar slope positions, aspects and slope gradients (~10°). The soil types in the studied plantations was loessial

soil with the texture of silty loam, consisting of clay, silt, sand was 4.31%, 59.63%, 36.06% for Mspa, 4.83%, 64.71%, 30.46% for Pp, 4.27%, 68.94%, 26.76% for Ps. The bulk density (0–20 cm) was 1.21, 1.17, 1.08 g/cm³ for Mspa, Pp, Ps, respectively. Moreover, the three types of plantations had similar plantation densities (~ 2000 tree. ha⁻¹). The mean diameter at breast height (DBH) measurements and average heights of the studied plantations are shown in [Table 1](#). Five individual plants of each species were randomly selected in each plot from May to September (growing seasons), 2016. The total precipitation and average temperature during the growing seasons of 2016 were 432.6 mm and 19.59 °C, respectively, and 91.35% of precipitation occurred during the growing season (Wang et al., 2017). Three xylem and leaf samples of each species were collected in each plot per month. The outer bark and phloem tissue were removed for the xylem samples, immediately placed into glass vials, sealed with parafilm and kept frozen (–20 °C).

Soil samples were collected simultaneously with a power auger around the sampled plants at a depth of 0–400 cm monthly from May to September 2016 (three samples each time). Soil core was collected every 10 cm in the 0–20 cm range, every 20 cm in the 20–120 cm range, every 30 cm in the 120–210 cm range, every 40 cm in the 210–250 cm range and every 50 cm in the 250–400 cm range. A total of 630 soil samples were collected during this study. The soil from each layer was mixed well and separated into two parts. One part was placed into glass vials, wrapped with parafilm and stored in a freezer (–20 °C). The other part was used to obtain the gravimetric soil water content (SWC, %) by drying at 105°C for 24 h. In addition, we also collected a

total of 46 rainwater samples.

Table 1 Characteristics of the three types of forest plantations.

Types	Species	Family	Average height (m)	Mean DBH (cm)
Mixed plantation (Mspa)	<i>R. pseudoacacia</i>	Leguminosae	7.18	6.32 ± 1.35
	<i>A. sibirica</i>	Rosaceae	6.84	5.58 ± 2.01
	<i>A. altissima</i>	Simaroubaceae	5.67	4.72 ± 1.62
Pure plantation (Pp)	<i>R. pseudoacacia</i>	Leguminosae	6.86	5.64 ± 2.86
Pure plantation (Ps)	<i>A. sibirica</i>	Rosaceae	6.22	6.33 ± 1.74

2.3 Stable isotope analysis and water source partitioning

The xylem and soil samples were extracted by a cryogenic vacuum distillation system (LI-2100, LICA, Beijing, China). The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of the xylem water were measured by using an isotope ratio mass spectrometer (IRMS) (MAT253, Thermo Fisher Scientific, Bremen, Germany). The precision was $\pm 1 \text{ ‰}$ for $\delta^2\text{H}$ and $\pm 0.2 \text{ ‰}$ for $\delta^{18}\text{O}$. The $\delta^{13}\text{C}$ in the plant leaf samples was analysed by IRMS, and the precision was $\pm 0.15 \text{ ‰}$. The isotopic compositions of the soil and rainfall water were measured by isotopic ratio infrared spectroscopy (IRIS) (DLT-100, Los Gatos Research, Mountain View, USA). The precision was $\pm 1.2 \text{ ‰}$ for $\delta^2\text{H}$ and $\pm 0.3 \text{ ‰}$ for $\delta^{18}\text{O}$ (Wang et al., 2009).

Soil water is the primary vegetation water source on the Loess Plateau because plant roots cannot reach the depth of groundwater, and there is no irrigation in the study area. Plant water source partitioning was determined by the Bayesian mixing model MixSIAR (version 3.1.7) (Stock and Semmens, 2013; Wang et al., 2019b). The raw isotopic ratios of the xylem water were input into MixSIAR as the mixture data. The averages and standard deviations of the soil water isotopes in the different soil layers

were the source data. The discrimination was set to zero for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ because there is generally no isotopic discrimination of water during plant water uptake by roots (Ehleringer and Dawson, 1992). The Markov chain Monte Carlo run length was set to 'long' (chain length = 300,000; burn = 200,000; thin = 100; chains = 3). For the subsequent analysis and comparison, the plant water sources were divided into shallow (0–80 cm), middle (80–210 cm) and deep (210–400 cm) layers according to the fluctuations and patterns of isotopic ratios in the soil water, SWC and the impact of rainfall pulse. (1) 0–80 cm soil layer: The variability of soil water isotopic compositions and SWC in this layer were larger, and was vulnerable to rainfall pulse and evaporation with seasons. (2) 80–210 cm soil layer: The variability of soil water isotopic compositions and SWC in this layer were lower than that of 0–80 cm soil layer. The impact of rainfall pulse and evaporation were moderate. (3) 210–400 cm soil layer: This layer had relatively stable variations in soil water isotopic compositions and SWC with seasons and soil depths.

2.4 Data analysis

A Kolmogorov–Smirnov (K–S) test was used to test whether the isotopic compositions of the plant tissues and soil water were normally distributed. Mixed linear and random slope models were applied to test for differences in the SWC and isotopic signatures in the soil and xylem water among the different plantation types. Soil depth, sampling time and plantation type were set as fixed effects, and plot was a random effect. In addition, differences in WUE_i and seasonal variation in water source partitioning were also tested by using mixed linear and random slope models. A critical

value of $p < 0.05$ was used to identify statistical significance. Statistical analyses were conducted using R software (v.3.4.4, R Core Team, 2018).

3. Results

3.1 Isotopic compositions of the soil and xylem water

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of soil water in the different plantation types varied with soil depth and season (Fig. 2). In Mspa, the average $\delta^{18}\text{O}$ value of soil water was $-8.88 \pm 1.75\text{‰}$ (mean \pm 1SD), and average $\delta^2\text{H}$ value was $-67.14 \pm 11.86\text{‰}$. The average $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of soil water in Pp were $-64.06 \pm 25.12\text{‰}$ and $-8.71 \pm 3.53\text{‰}$, respectively. The average isotopic compositions of soil water in Ps were $-67.78 \pm 12.57\text{‰}$ for $\delta^2\text{H}$ and $-8.66 \pm 2.13\text{‰}$ for $\delta^{18}\text{O}$. The soil water isotopes exhibited clear variations along the soil profile. The shallow soil water displayed more enriched isotopic values and more variation with season. The deep soil water showed more depleted isotopic values with depth and less variation with season. The isotopic ratios of soil water in each plantation type were statistically different ($p < 0.001$) among seasons and depths. However, there was no significant difference ($p=0.052$ for $\delta^2\text{H}$, $p=0.61$ for $\delta^{18}\text{O}$) in the soil isotopic compositions in the different plantation types.

The isotopic compositions of xylem water changed with season and species. In Mspa, the average isotopic ratios of xylem water were $-8.41 \pm 0.72\text{‰}$ for $\delta^{18}\text{O}$ and $-65.57 \pm 4.37\text{‰}$ for $\delta^2\text{H}$ in *R. pseudoacacia*, $-7.21 \pm 1.42\text{‰}$ for $\delta^{18}\text{O}$ and $-59.68 \pm 7.42\text{‰}$ for $\delta^2\text{H}$ in *A. sibirica*, and $-7.72 \pm 0.89\text{‰}$ for $\delta^{18}\text{O}$ and $-64.53 \pm 4.56\text{‰}$ for $\delta^2\text{H}$ in *A. altissima*. In Pp, the isotopic ratios of xylem water ranged from -9.35 to -5.98‰ for

$\delta^{18}\text{O}$ and -75.36 to -55.68‰ for $\delta^2\text{H}$. The average isotopic signatures of xylem water in Ps were $-7.20 \pm 1.33\text{‰}$ for $\delta^{18}\text{O}$ and $-61.49 \pm 6.25\text{‰}$ for $\delta^2\text{H}$. The xylem water isotopes in different species in Mspa were significantly different ($p < 0.001$). In addition, the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of xylem water exhibited significant variation with season ($p < 0.001$). Most of the soil water isotopes were on the right of the local meteoric water line (LMWL), and the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of xylem water were within the range of soil water isotopes (Fig. 3), which implied the plants predominantly obtained water sources from different soil layers.

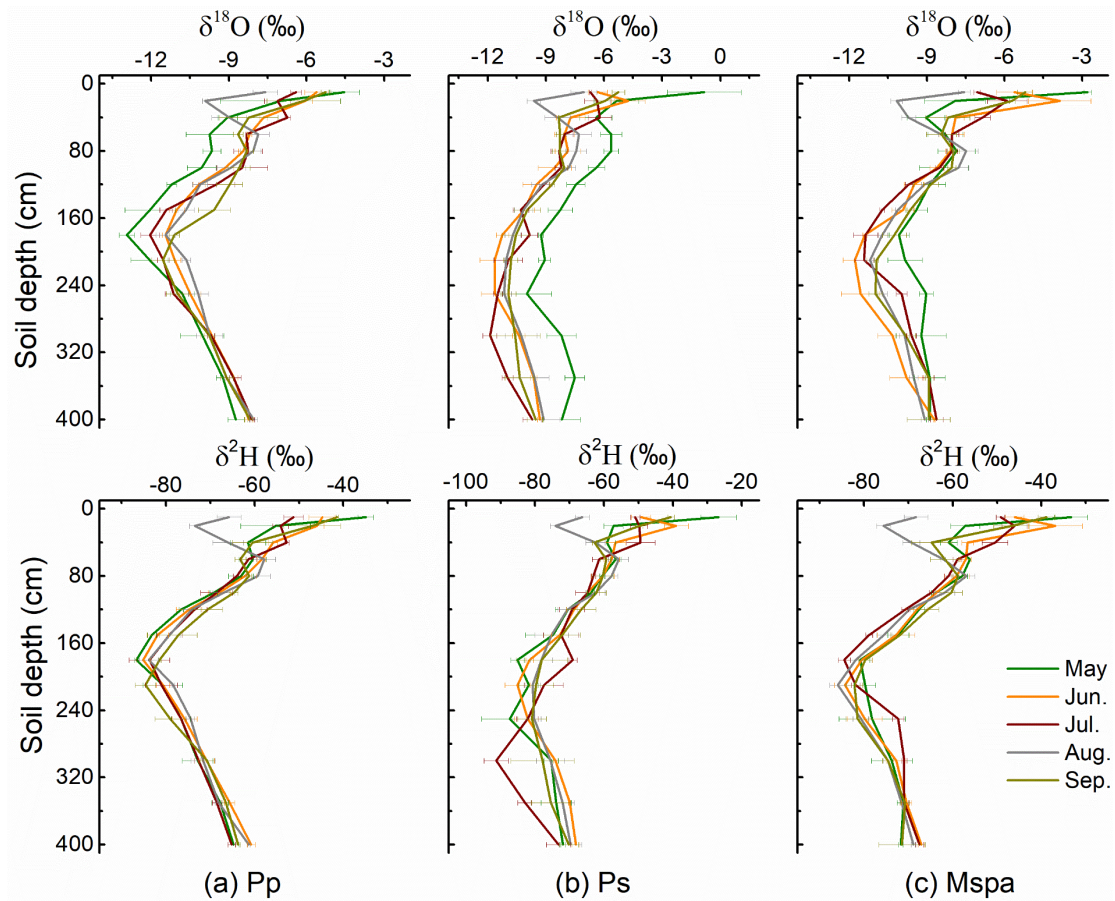


Fig. 2. Seasonal variations of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in soil water along the soil profile (0-400 cm) in (a) Pp (b) Ps (c) Mspa from May to September. Data are expressed as the means \pm 1SD.

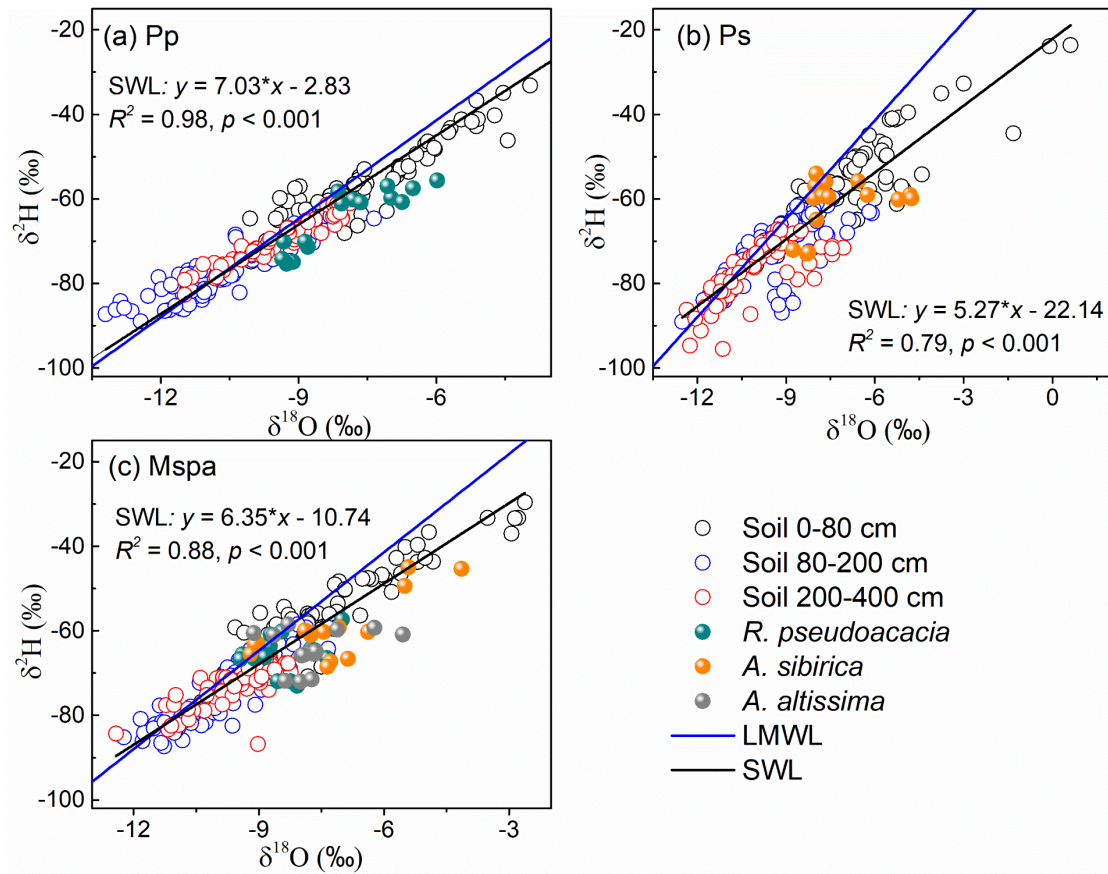


Fig. 3. The relationships of isotopic values in the xylem water and soil water from three soil layers in the different plantation types, (a) Pp, (b) Ps and (c) Mspa, during the study periods. LMWL is the local meteoric water line ($y = 7.76x + 5.14$, $R^2 = 0.91$, $p < 0.01$). The soil water evaporation line (SWL) was fitted based on the soil water isotopic values.

3.2 Soil water availability and plant water source partitioning

The SWCs of the different plantation types displayed clear seasonal and vertical variations (Fig. 4). The average SWCs were $7.01 \pm 1.70\%$ in Mspa, $6.68 \pm 1.46\%$ in Pp, and $7.13 \pm 2.19\%$ in Ps during the study periods. The SWC of the shallow soil water was highest in Mspa, and the deep soil moisture in Pp had the lowest values during the study periods. The shallow soil moisture showed relatively higher fluctuations with season, while the deep soil moisture varied less with season. The SWCs of both the

shallow and middle soil layers in the different plantation types showed no significant differences. However, the deep soil moisture differed significantly in the different plantation types ($p < 0.001$). In addition, the SWCs of the three plantation types showed significant differences among seasons ($p < 0.01$). Overall, a significant difference in soil moisture was found among the different plantation types ($p < 0.05$).

Plants mainly took up shallow and mid-layer soil moisture throughout the growing seasons (Fig. 5). *R. pseudoacacia* in Mspa obtained 74.86% of its water from the shallow and middle soil layers, and *A. altissima* in Mspa obtained 75.62% of its water from the shallow and middle soil layers during the study periods. *A. sibirica* in Mspa obtained the largest proportion of shallow soil water (60.96%) during the sampling periods. The proportional contributions of middle and deep soil water obtained by *R. pseudoacacia* in Pp during the whole growing season were 32.88% and 27.14%, respectively. *A. altissima* in Ps derived 43.58% of its water from the shallow soil layer and 32.12% of its water from the middle soil layer during the study periods. In Mspa, the water uptake proportions from different soil layers had pronounced differences among the three species and months ($p < 0.05$). The proportions of water sources used by *R. pseudoacacia* in Mspa and *R. pseudoacacia* in the pure plantations from different soil layers exhibited no significant difference. However, *A. sibirica* in Mspa and *A. sibirica* in the pure plantation had a pronounced difference in the proportions of shallow soil water use ($p < 0.05$), but there was no significant difference in the proportions of water sources from the deep and middle soil layers. In addition, the proportional contributions of shallow, middle and deep soil water for *A. altissima* in Mspa and *A.*

sibirica in the pure plantation had no significant differences among seasons.

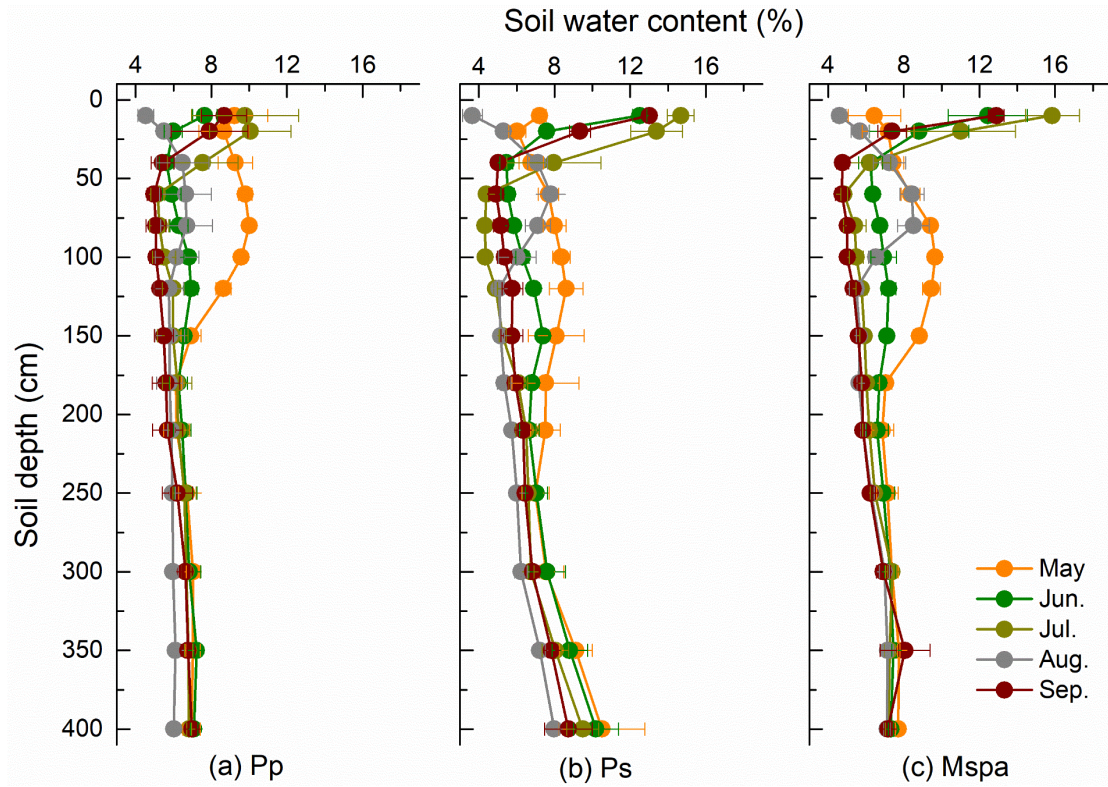


Fig. 4. Seasonal variation in gravimetric soil water content (%) in different soil layers from (a) Pp (b) Ps, and (c) Mspa. Data represent the means \pm SD.

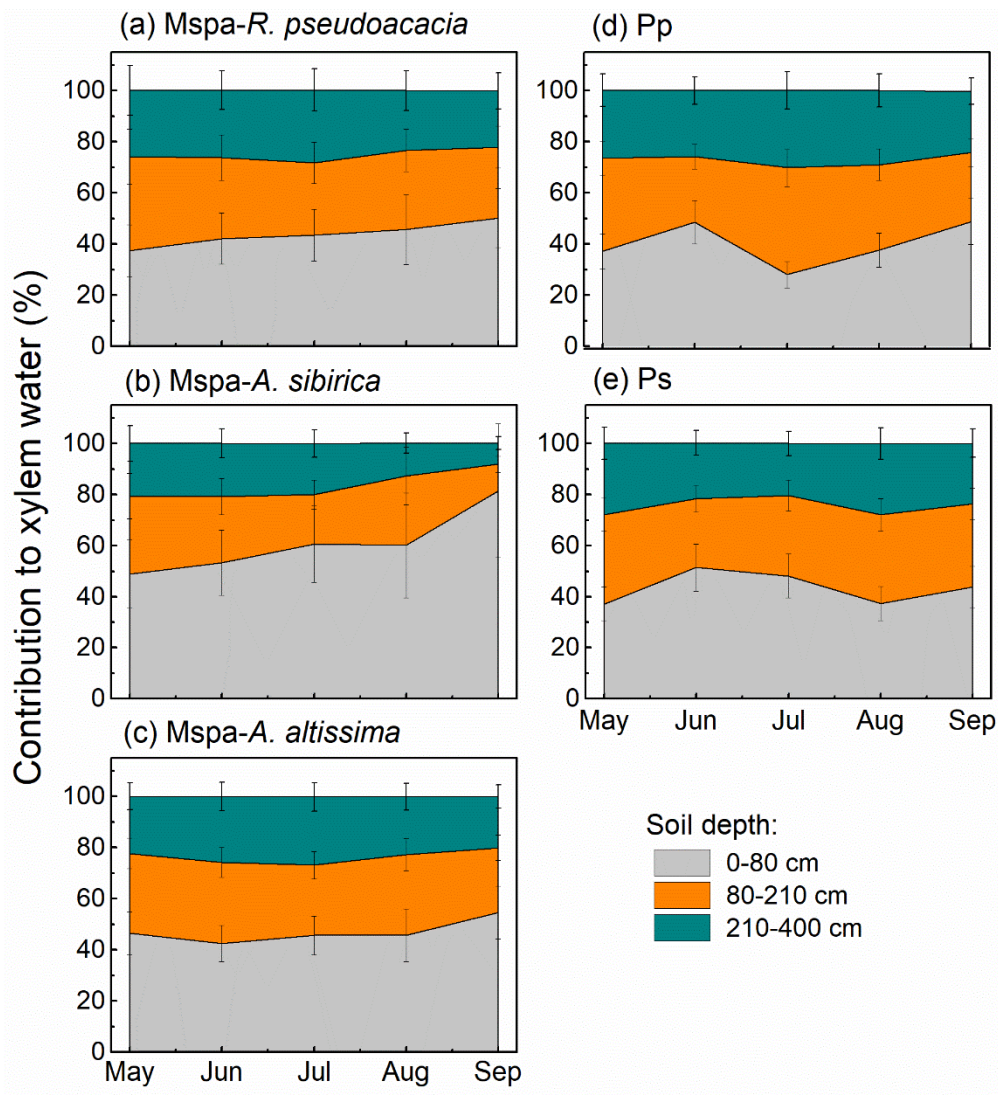


Fig. 5. Seasonal variation in the contributions of water from different soil layers to the xylem water in the different plantation types, (a) Mspa-R. *pseudoacacia*, (b) Mspa-A. *sibirica*, (c) Mspa-A. *altissima*, (d) Pp, (e) Ps and, based on the MixSIAR model. Error bars represent the standard deviation.

3.3 Plant leaf $\delta^{13}\text{C}$ values

The $\delta^{13}\text{C}$ values in the plant leaves varied with season and plant species during the sampling periods (Fig. 6). The average values of $\delta^{13}\text{C}$ in the plant leaves of *R. pseudoacacia*, *A. sibirica* and *A. altissima* in Mspa were $-26.77 \pm 0.58\text{‰}$, $-26.28 \pm 0.54\text{‰}$

and $-26.64 \pm 0.75\%$, respectively. The plant leaf $\delta^{13}\text{C}$ value of *A. sibirica* in Ps was lower ($-27.47 \pm 0.15\%$) than that in the mixed plantation. *R. pseudoacacia* in Pp had the lowest plant leaf $\delta^{13}\text{C}$ value ($-28.00 \pm 0.80\%$) of all study plants during the sampling periods. Overall, the plant leaf $\delta^{13}\text{C}$ values differed significantly among species and seasons ($p < 0.05$). In the mixed plantation, the plant leaf $\delta^{13}\text{C}$ value of *A. sibirica* was very different from those of *R. pseudoacacia* and *A. altissima* and displayed significant seasonal variation. In addition, there was a significant difference ($p < 0.001$) between the $\delta^{13}\text{C}$ in the plant leaves of *R. pseudoacacia* in Mspa and *R. pseudoacacia* in the pure plantation during the study periods. The $\delta^{13}\text{C}$ value of the leaves of *A. sibirica* in Mspa and *A. sibirica* in the pure plantation was also significantly different ($p < 0.001$) during the sampling periods.

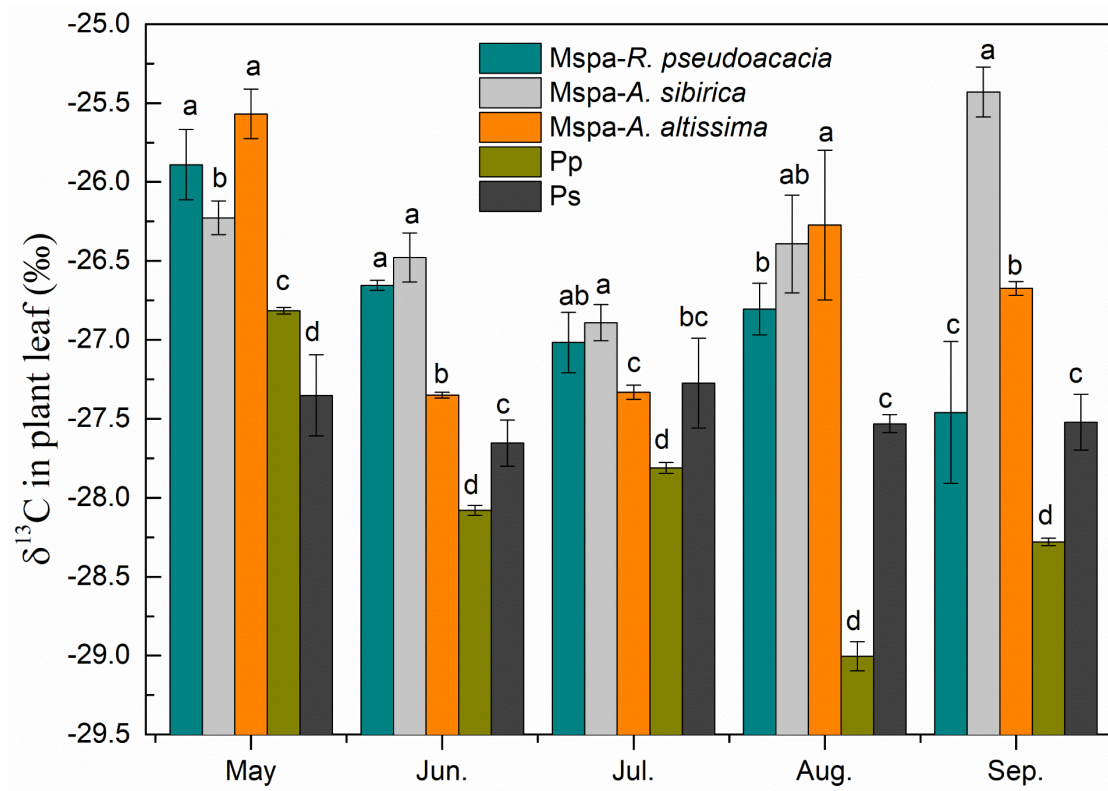


Fig. 6. Seasonal variations in the plant leaf $\delta^{13}\text{C}$ values in the different plantation types from May

to September. Error bars represent the standard deviation.

3.4 Relationship between plant leaf $\delta^{13}\text{C}$ values and SWCs

The SWCs including all data from shallow, middle and deep soil layer under different plantations were correlated to the $\delta^{13}\text{C}$ values in plant leaves of the species under corresponding plantations during the study periods (Fig. 7). The relationship between SWCs and the plant leaf $\delta^{13}\text{C}$ values of *R. pseudoacacia* in Mspa were inconsistent with that of *R. pseudoacacia* in Pp. Although the $\delta^{13}\text{C}$ values in plant leaves of *R. pseudoacacia* under both the mixed and pure plantation were positively associated with SWCs, weaker associations were found in the mixed plantations compared with pure plantation ($R^2=0.25$, $p=0.06$ for *R. pseudoacacia* in Mspa, and $R^2=0.53$, $p=0.002$ for *R. pseudoacacia* in Pp) (Fig. 7). However, this relationship was not observed for *A. altissima* in different plantations during the sampling periods. The $\delta^{13}\text{C}$ values in plant leaf of *A. altissima* and SWCs showed no significant correlation for both the mixed and pure plantations. In addition, the plant leaf $\delta^{13}\text{C}$ values of *A. altissima* in Mspa was larger than that in the pure plantation for a given SWC.

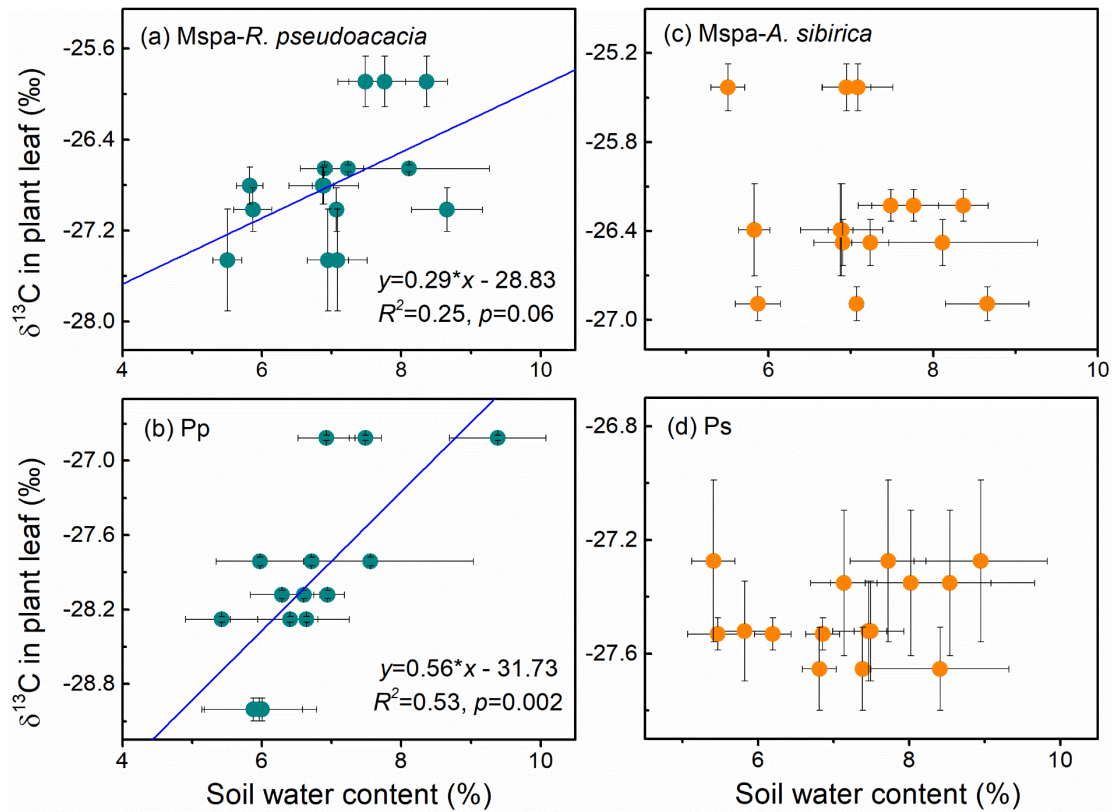


Fig. 7. The relationship between soil water content (SWC, %) and $\delta^{13}\text{C}$ in the plant leaf of the species in different plantation types, (a) *Mspa-R. pseudoacacia*, (b) *Pp*, (c) *Mspa-A. sibirica*, (d) *Ps*.

The monthly average SWCs and plant leaf $\delta^{13}\text{C}$ values were used in the fitting. Error bars represent the standard deviation.

4. Discussion

4.1 Water source partitioning in the mixed plantation

The xylem water isotopes of the three plant species in Mspa were significantly different ($p < 0.001$), suggesting that the three plant species had distinct water sources. In addition, the MixSIAR outcomes also indicated that the proportional contributions of water from different soil layer for the three plant species in Mspa had pronounced differences ($p < 0.05$) (Fig. 5). These results suggest that the three coexisting plants in

the mixed plantation exhibited water source segregation. This result was inconsistent with a previous study in the similar study area (Tang et al., 2018) which found the plants species in the mixed stands had overlapping water sources and enhanced water competition. This discrepancy may be due to the species-specific hydrological niche. Some previous studies suggested that coexisting plants adopt water source segregation to promote plant coexistence (Meißner et al., 2012; Silvertown et al., 2015). Hydrological niche segregation has been considered the primary reason for plant coexistence (Martorell et al., 2015; Silvertown et al., 2015). Water availability is a limiting factor controlling vegetation structures in arid and semi-arid ecosystems, and plants usually resist the arid environment through hydrological niche segregation. Soil and plant properties are crucial factors affecting plant water use. Soil variabilities such as soil texture, bulk density, affected water holding capacity and migration along with soil profiles (Yang et al., 2017), and then regulated plant water uptake. Soil properties in the mixed stand are consistent for the three plant species. Therefore, soil properties are not responsible for water source segregation of the three species in the mixed plantations. In addition, plant attributes (such as fine root distribution, heights and stem diameters) played a critical role in affecting plant water use patterns. In our study, the three deciduous species in Mspa had similar heights and stem diameters. A previous study suggested *A. sibirica* has a wider lateral root distribution than *R. pseudoacacia* (Jian et al., 2015). For this reason, *A. sibirica* in Mspa derived a higher proportion of shallow soil water (60.96%) than *R. pseudoacacia* in Mspa (43.72%) during the growing seasons. Moreover, coexisting plants adjust their water uptake

patterns, which minimizes water competition through physiological adjustments (Quero et al., 2011; Tang et al., 2018). Quero *et al.* (2011) showed that plant species adjusted their hydraulic strategies from isohydric to an-isohydric in response to drought stress in six coexisting Mediterranean woody species. Some plant species adopt stomatal control, leaf area adjustment and photosynthesis recovery after wilting to enhance plant species coexistence (Angert et al., 2009; Renninger et al., 2015; Swemmer et al., 2006).

The leaf $\delta^{13}\text{C}$ of *A. sibirica* was significantly higher than those of *R. pseudoacacia* and *A. altissima* in Mspa, indicating that *A. sibirica* had a higher leaf-level intrinsic water use efficiency (WUE_i) than *R. pseudoacacia* and *A. altissima* in Mspa during the study periods. A previous study found that leaf $\delta^{13}\text{C}$ in C_3 plants was positively correlated with leaf-level WUE_i (Moreno-Gutierrez et al., 2012). Plants with a high WUE_i (e.g., *A. altissima*) in coexisting communities were likely more competitive at coping with drought conditions. In our study, *A. sibirica* in Mspa mainly relied on shallow soil water and had a high WUE_i . This result was inconsistent with a previous study in a Mediterranean ecosystem (Moreno-Gutierrez et al., 2012), where coexisting plants with low WUE_i and high stomatal conductance showed relatively more opportunistic water use patterns and depended heavily on shallow soil water. This discrepancy may be caused by eco-physiological niche segregation.

4.2 Water use characteristics in the different plantation types

The plants in the pure plantations displayed distinct water uptake partitioning compared with those in the mixed plantations (Fig. 5, 6). There was no significant

difference in the water uptake proportions of *R. pseudoacacia* between the different plantation types. However, the proportional contributions of shallow soil water to *A. sibirica* in the different plantation types were significantly different. This relationship was dependent on soil depth, and a significant difference was observed in the shallow soil water layer but not in the middle and deep soil water layers. This result suggested that plantation types affected plant water source partitioning and that a species-specific response to the plantation type existed. Tang *et al.* (2018) also reported that mixed afforestation had a significant effect on the water sources of plant species on the semiarid Loess Plateau. The plant leaf $\delta^{13}\text{C}$ values of both *R. pseudoacacia* and *A. sibirica* differed significantly among plantation types, suggesting plantation types affected plant leaf-level WUE_i . In addition, the leaf $\delta^{13}\text{C}$ of *A. sibirica* in Ps was significantly higher than that of *R. pseudoacacia* in Pp (Fig. 6), suggesting that *A. sibirica* had a higher leaf-level WUE_i than *R. pseudoacacia* in the pure plantation during the study periods. The plant leaf $\delta^{13}\text{C}$ value of *A. sibirica* was significantly different from that of *R. pseudoacacia* in the mixed plantation. These findings imply that *A. sibirica* and *R. pseudoacacia* in the different plantation types exhibited distinct water use characteristics. This discrepancy may be attributed to their different eco-physiological characteristics, such as different lateral root distributions (Jian *et al.*, 2015), specific leaf areas (Yan *et al.*, 2010), plasticity of minimum leaf water potential (Miyazawa *et al.*, 2018), and osmotic adjustment ability (Yan *et al.*, 2013). In the same catchment, Wei *et al.* (2018) reported that *R. pseudoacacia* was negatively affected by water stress but *A. sibirica* was not by comparing inter-annual radial growth responses

to climate change using tree-ring samples in the same catchment. A previous study also reported that *R. pseudoacacia* was drought-sensitive, with a higher water demand under water-limited conditions, but *A. sibirica* was not, with a lower sensitivity of stomatal regulation, by comparing the sap flow characteristics and climatic responses of the two species (Du et al., 2011).

The relationship between SWCs and the plant leaf $\delta^{13}\text{C}$ values displayed inconsistent pattern among plant species and plantations (Fig. 7). The $\delta^{13}\text{C}$ values in plant leaves of *R. pseudoacacia* under different plantation were positively associated with SWCs, but the significant relationship was not observed in *A. sibirica*. These results indicated that water competition between different species in the mixed plantation has different effects than that between individuals of the same species in the pure plantation and this effect existed a species-specific. A possible explanation was that the interspecific competition in the mixed plantation makes each tree species establish different hydrological niches for water uptake (González de Andrés et al., 2017). *R. pseudoacacia* is a fast-growing tree species with drought-sensitive (Wei et al., 2018) and more water was demand to satisfy fast plant growth in the arid environment. Therefore, *R. pseudoacacia* displayed opportunistic water uptake strategy, which was reflected that the leaf-level WUE_i increased with SWCs (Fig. 7). However, *A. sibirica* is less sensitive to water stress and has a stronger ability to cope with drought stress (Wei et al., 2018). Moreover, the plant species evolutionary history may result in the diversity of water use strategies in the semiarid ecosystems (Moreno-Gutierrez et al., 2012). Soil properties in different plantations stands may also contribute to the diversity

of plant water use strategies, which needs to be further explored in future studies.

4.3 Implications

The climate of the Loess Plateau has become warmer and drier in the past 50 years (Wang et al., 2012b) and this trend may continue in the future according to the predictions of climate models (Huang et al., 2017). A warmer and drier climate combined with large-scale afforestation will aggravate water shortages on the Loess Plateau. The results here showed that plantation type significantly altered plant water use behaviour. One aspect was that the leaf-level WUE_i , significantly improved in the mixed plantation, which was detected by the plant leaf $\delta^{13}C$ (Fig. 6). Another aspect was that plantation type changed the plant water use patterns (Fig. 5). Our result indicated plantations types changed the effect of interspecific versus intraspecific competition for water source, and the plant species existed a species-specific response to this effect. *A. sibirica* in the mixed plantation derived a higher proportion of its water from the shallow soil than that in the pure plantation, and deep soil water depletion was correspondingly reduced. The SWC in the deep soil layer in the Mspa plot was significantly higher than that of *R. pseudoacacia* in Pp and lower than that of *A. sibirica* in Ps, suggesting that deep soil water consumption was reduced in the mixed plantation to a certain extent. This discrepancy was only observed in the deep soil layer and not in the shallow and middle soil layers. Moreover, Mixed afforestation alleviated the occurrence of soil desiccation in the Loess Plateau. However, these results do not necessarily mean that mixed plantations are suitable for revegetation in water-limited

areas. Although the presence of water source segregation among plants species in mixed forests, interspecific competition for water source may still occur especially during drought periods, causing serious environmental issues (e.g., tree mortality and soil desiccation). A previous study demonstrated that mixed afforestation induced water competition between plant species and the risk of carbon starvation and xylem embolism despite physiological trait adjustments (Tang et al., 2018). In addition, a warmer and drier climate on the Loess Plateau will undermine the persistence of revegetation in ecological restoration (Gao et al., 2018). The unprecedented change in soil water availability may alter plant water use characteristics and ecosystem stability under climate change.

In this study, we only investigated the water use strategies of tree species in different plantation types. The water use characteristics of plant species of other mixed types on the Loess Plateau, such as mixed grass-shrub and mixed tree-shrub types, should be investigated in future studies. In addition, ecohydrological separation hypothesis need to be evaluated to explore soil-plant-water interactions in vegetation restoration. Using different plant densities and applying field management strategies are also important for effectively restoring vegetation in semiarid ecosystems because these factors alter the persistence of revegetation and the interacting relationships among coexisting species (Oliva Carrasco et al., 2015; Tschardtke et al., 2011).

5. Conclusion

In this study, a stable isotope technique was applied to detect the seasonal water

use characteristics of plant species in different plantation types on the semiarid Loess Plateau by measuring the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of xylem and soil water and plant leaf $\delta^{13}\text{C}$. The results showed that the three coexisting tree species had significant difference ($p < 0.05$) in the proportional contribution of water from different soil layer, implying that these species had hydrological niche segregation. The contributions of soil water for *R. pseudoacacia* in the different plantation patterns showed no significant difference. However, the contributions of shallow soil water for *A. sibirica* in the different plantation types were significantly different, but no differences were found for the middle and deep soil water layers. The leaf $\delta^{13}\text{C}$ values of the plant species in the mixed plantation were significantly higher than those of the plant species in the pure plantations, which implied that the leaf-level WUE_i significantly improved in the mixed plantation. In addition, *A. sibirica* in the mixed plantation obtained more water from the shallow soil than that in the pure plantation, and correspondingly, deep soil water consumption was reduced. These findings suggest that plantation types affected plant water source partitioning, and species-specific responses to the plantation type existed. This study provides important baseline information and insights into afforestation and ecological management related to water use in arid and semiarid ecosystems.

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