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Use of ^2H and ^{18}O stable isotopes to investigate water sources for different ages of *Populus euphratica* along the lower Heihe River

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Abstract Investigation of the water sources used by trees of different ages is essential to formulate a conservation strategy for the riparian tree, *P. euphratica*. This study addressed the contributions of different potential water sources to *P. euphratica* based on levels of stable oxygen and hydrogen isotopes ($\delta^{18}\text{O}$, $\delta^2\text{H}$) in the xylem of different aged *P. euphratica*, as well as in soil water and groundwater along the lower Heihe River. We found significant differences in $\delta^{18}\text{O}$ values in the xylem of different aged *P. euphratica*. Specifically, the $\delta^{18}\text{O}$ values of young, mature and over-mature forests were $-5.368(\pm 0.252)\text{‰}$, $-6.033(\pm 0.185)\text{‰}$ and $-6.924(\pm 0.166)\text{‰}$, respectively, reflecting the reliance of older trees on deeper sources of water with a $\delta^{18}\text{O}$ value closer to that of groundwater. Different aged *P. euphratica* used different water sources, with young forests rarely using groundwater (mean $< 15\%$) and instead primarily relying on soil water from a depth of 0–50 cm (mean $> 45\%$), and mature and over-mature forests using water from deeper than 100 cm derived primarily from groundwater.

Keywords Water sources · *Populus euphratica* · ^2H and ^{18}O stable isotopes · Heihe River · Tree age

Introduction

P. euphratica (Euphrates poplar) is widely distributed in inland river basins of arid zones in northwest China. This tree can tolerate salinity, drought and wind and has important ecological benefits (Chen et al. 2004). The Food and Agriculture Organization has identified *P. euphratica* as an urgent priority for protection of forests genetic resources; however, a detailed understanding of their water source is essential to their conservation.

Plant water sources can be determined through stable isotope analysis of stem water (Dawson et al. 2002; Asbjornsen et al. 2007; Hasselquist et al. 2010). There is usually no isotopic fractionation during water uptake by terrestrial plants, although examples of plant species that seem to fractionate hydrogen (but not oxygen) during water uptake do exist (Ellsworth and Williams 2007). Water sources can be pinpointed by comparing the isotope ratios of all potential sources with those of water extracted from plant stems (Dawson et al. 2002).

Water sources differ greatly between and within species. Sources used by an individual plant may vary greatly over time, especially in arid regions (Nie et al. 2012). Plant roots interact with soil texture, water availability and salinity to determine which sources are most easily used by the plant (Overdieck et al. 2013). In species capable of growing to a large size, root distribution may vary greatly with plant age (Dawson 1996).

Because *P. euphratica* is the foundation species of inland riparian forests, its ecological characteristics, stress resistance and water use have been the focus of much research (Chen et al. 2005; Li et al. 2008; Chen et al. 2011; Yang et al. 2012). Studies using the stable isotopes ^2H and ^{18}O have shown that sand dune plants in the middle reaches of the Heihe River Basin occasionally use rainwater, but primarily rely on the more stably available phreatic water (Zhou et al. 2011; Yu et al. 2012). Studies in the lower Heihe River Basin have shown that the growth of woody plants mainly depends

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on groundwater, while herbs rely on surficial soil water (Zhao et al. 2008). Along the lower reaches of the Tarim River, groundwater is the main water source for survival of *P. euphratica* (Chen et al. 2006). In this study, we addressed water sources used by different aged *P. euphratica* in the lower reaches of the Heihe River Basin. We used stable isotopes of ^2H and ^{18}O and analysis of soil moisture to assess the contributions of rainfall and groundwater for young, mature and over-mature forests. Our goal was to provide a scientific basis for ecological water configuration measures designed to protect *P. euphratica* in the lower Heihe River Basin.

Data and methods

Study area

Our study area is located in the lower reaches of the Heihe River, within Ejin Banner, Inner Mongolia, China (Fig. 1). The region is characterized by a dry, continental climate. The average annual precipitation from 1960 to 2010 was 42 mm, with maximum and minimum values of 103 mm and 7 mm, while the mean annual potential evapotranspiration (PET) was 3755 mm, with a maximum of 4035 mm. Therefore, the potential evapotranspiration was 89 times the rainfall. The mean annual temperature was 8.2 °C. The prevailing north-west winds have an average speed of 4.2 m/s, and a maximum of 24.0 m/s. The riparian vegetation is dominated by *P. euphratica*, as well as the shrubs *Tamarix ramosissima* and *Sophora alopecuroides*.

Sample collection

The study site is located on the flood plain of the Heihe River near Ulan Tug (Fig. 1). There has been no inundation for many years, and there is no significant ground-surface slope. One representative plot was selected within each of three age classifications of *P. euphratica*: young forests, mature forests and over-mature forests. Three individuals within each plot that met the following requirements were selected: good growth, straight trunk, moderate crown, and no plant diseases or insect pests. A plant stem sample was taken from each selected individual, and a nearby pit was augured to the water table for soil moisture determination. All plots were sampled during August 2012. No rain or water condensation occurred at the study area within a few days before or after sampling. Age group classifications were made according to the standard practice in the forestry sector. Plot characteristics are shown in Table 1, and groundwater parameters are shown in Table 2.

Determination of soil moisture

Soil moisture was measured in vertical layers, with three samples per layer. Layers were 10 cm thick between 0 and 40 cm and 20 cm thick at depths greater than 40 cm. Measurements extended down to the water table, 260 cm in over-mature forests and 320 cm in young and mature forests. Each sample was immediately weighed in the field and then reweighed in the lab after oven-drying at 105 °C to a constant mass.

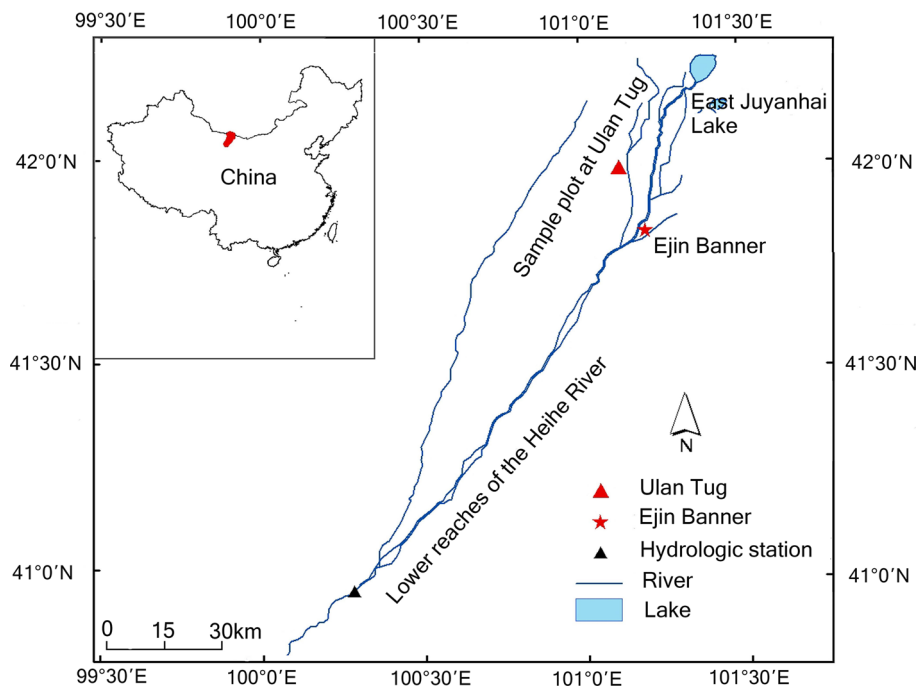


Fig. 1 Map of the study area

Table 1 Ecosystem characteristics of different ages of *Populus euphratica* forests near Ulan Tug

Characteristic	Young forests	Mature forests	Over-mature forests
Height of tree (m)	3.5	12.9	14.7
Diameter at breast height (cm)	7	65	112
Crown (m × m)	1.2 × 1.5	6.7 × 7.5	10.3 × 11.6
Age (a)	4–6	30–40	55–60
Groundwater level (cm)	320	320	260
Soil texture	0–110 cm were sandy loam, 110–130 were clay, 130–320 were sandy soil	0–70 cm were sandy loam, 70–90 were clay, 90–180 were sandy soil, 180–200 were clay, 200–320 were sandy soil	0–110 cm were sandy loam, 110–130 were clay, 130–250 were sandy soil.
Root distribution(cm)	20–40	60–120	60–160
Understory vegetation	<i>Sophora alopecuroides</i> , <i>Peganum harmala</i> , <i>Herba Taraxaci</i>	Thin <i>Sophora alopecuroides</i>	Minimum <i>Sophora alopecuroides</i>
Coverage	0.7	0.5	0.3

Table 2 Groundwater parameters of sample

Groundwater parameters	Values
Total alkalinity (me L ⁻¹)	3.896
PH	7.91
Salinity (g L ⁻¹)	0.814
Total hardness (me L ⁻¹)	7.500
Cl ⁻ (g L ⁻¹)	0.082
Total salt (g L ⁻¹)	0.812

Xylem and soil water isotope sampling

Stems selected for xylem sampling were more than 2 years old with a diameter of 0.3–0.5 cm and a length of 3–5 cm. The bark, phloem and cambium were removed to expose the xylem, which was quickly loaded into glass bottles, covered with a cork, sealed with parafilm, and placed on ice in a cooler for transport back to the lab. Soil water samples were collected for isotope analysis following the protocol for soil moisture. Groundwater samples were taken from groundwater observation wells.

Laboratory methods

Previous studies have demonstrated that there is no isotope fractionation in plants during water transport from roots to leaves through stems, except in salt-excluding plants in coastal wetlands (Dawson et al. 1991; Zhao et al. 2008). Therefore, as long as the stable isotope compositions of the potential water sources are significantly different, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of xylem water and each water source can be used to determine the relative contribution of each source (Dawson et al. 1991; Busch et al. 1992; Dawson et al. 1993; Phillips et al. 2001; Zhang et al. 2004; Asbjornsen et al. 2007).

In this study, we compared the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ from xylem water and soil water with the goal of determining from where in the soil profile *P. euphratica* obtains its water. Values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in soil water decreased

monotonically with increasing depth, reflecting the change in water source from precipitation to groundwater; therefore, we used values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ to calculate the relative contributions of soil water and groundwater for any age and depth.

Water in the soil and plant stem samples was extracted cryogenically using the approach described by Ehleringer et al. (2000). An LGR liquid water isotope analyzer was used to measure hydrogen and oxygen isotope ratios of groundwater, xylem water and soil water in each layer.

$$\delta^2\text{H}(\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000 \quad (1)$$

$$\delta^{18}\text{O}(\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000 \quad (2)$$

$\delta^2\text{H}$ and $\delta^{18}\text{O}$ represent hydrogen and oxygen isotope values of samples, while R_{sample} and R_{standard} are the stable isotopic compositions (i.e. the $^2\text{H}/\text{H}$ molar ratio) of the sample and the standard water (SMOW; Ehleringer et al. 2000). The accuracy (1σ) was 0.1 ‰ for $^{18}\text{O}/^{16}\text{O}$ and 0.3 ‰ for $^2\text{H}/\text{H}$. The test error was less than 1 ‰ for $\delta^2\text{H}$ and less than 0.2 ‰ for $\delta^{18}\text{O}$.

The IsoSource mixing model (<http://www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm>) (Phillips et al. 2003) used stable isotope values to determine the relative contributions of soil water and groundwater as a function of depth. The user supplies the source increment (e.g., entering a value of 1 % specifies examination of all possible combinations of sources contributions from 0 to 100 % in increments of 1 %), and the mass balance tolerance (e.g., entering a value of 0.1 l specifies that all source combinations that result in predicted mixture signatures within 0.1 l of the observed signature are considered feasible solutions). IsoSource provides output files that list each feasible solution, descriptive statistics about the distribution of these solutions (number of solutions, mean, standard deviation, minimum, maximum, 1st percentile, median, and 99th percentile for each source), and histograms of these distributions. Statistical analyses were performed with SPSS17.0 and plotted with origin 8.0.

Results

Soil moisture

Soil moisture profiles varied with age of *P. euphratica* (Fig. 2). Overall, soil moisture was higher in young forests than mature or over-mature forests. At a finer scale, the soil moisture profile could be divided into 3 zones, 0–100 cm, 100–200 cm and 200 cm to the saturated zone. At depths of 0–100 cm, the soil moisture ranges were 3.86–18.89 % in young forests (Fig. 2a), 0.64–2.08 % in mature forests (Fig. 2b) and 2.09–4.15 % in over-mature forests (Fig. 2c).

All forests age groups displayed a peak in soil moisture between 100 and 200 cm. For young forests, a peak of 17.35 % occurred at 120–140 cm, while for mature forests, a peak of 7.08 % occurred at 180–200 cm and for over-mature forests a peak of 6.34 % occurred at 100–120 cm. Soil texture profiles did not differ among age groups; therefore, peaks in soil moisture may be related to the distribution of *P. euphratica* roots. In other words, soil moisture may be more abundant in young forests than mature or over-mature forests because young trees consume less water (Li et al. 2008). As expected soil moisture increased strongly near the water table (Fig. 2), with peak values for young, mature and over-mature forests of 31.8, 24.6 and 28.6 %, respectively. The *P. euphratica* root distribution was more shallow in young forests (20–40 cm) than in mature forests (60–120 cm) or over-mature forests (60–160 cm) (Table 1).

Water absorption as a function of age and depth

For all three age classifications, the shallowest soil water sample, 5 cm in depth, had the highest or near highest

measured value of $\delta^{18}\text{O}$ (4.4, 4.1 and 1.5 ‰ for young forests, mature forests and over-mature forests, respectively). Values of $\delta^{18}\text{O}$ decreased with increasing depth, with the greatest rates of decrease occurring near the surface, especially in young forests, reflecting evaporation of soil water near the surface. In young forests, $\delta^{18}\text{O}$ values decreased rapidly from 4.6 ‰ to -3.4 ‰ from 0 to 30 cm. In contrast, from 60 cm to 320 cm, no trends in $\delta^{18}\text{O}$ values were observed, forests with all being close to the groundwater $\delta^{18}\text{O}$ value of -6.9 ‰. The decrease in $\delta^{18}\text{O}$ values in mature forests occurred at a greater depth than in young forests. Values of $\delta^{18}\text{O}$ fluctuated around 4 at depths of 0–60 cm, then dropped to -3.6 ‰ at 120 cm. At depths of 120–200 cm, the $\delta^{18}\text{O}$ values fluctuated around -5 ‰, while at 200–320 cm they were between -7.1 and -7.4 ‰, which was close to the groundwater $\delta^{18}\text{O}$ value (-6.9 ‰). Over-mature forests showed the same pattern of decreasing values of $\delta^{18}\text{O}$ with increasing depth, but the gradient was not as strong. The value near the surface was 1.5 ‰, while it decreased to -6.1 ‰ at 100 cm. From 120 to 260 cm, $\delta^{18}\text{O}$ values fluctuated around the value for groundwater. The similarity between deep soil and groundwater values of $\delta^{18}\text{O}$ for all forests ages strongly suggests that the deep soil water is derived from groundwater.

Because xylem values of $\delta^{18}\text{O}$ should equal the volume-weighted average of all the sources used by the tree, comparison of soil and xylem values can suggest from where roots are deriving their water. In young forests, soil water and xylem water values of $\delta^{18}\text{O}$ were most similar at depths of 30–50 cm, suggesting that young forests is deriving water from shallow layers (Fig. 3a). In mature forests, $\delta^{18}\text{O}$ values of soil and xylem water were most similar at depths of 120–200 cm (Fig. 3c), suggesting that mature forests uses deeper water than young forests. In over-mature forests, $\delta^{18}\text{O}$ values of soil and

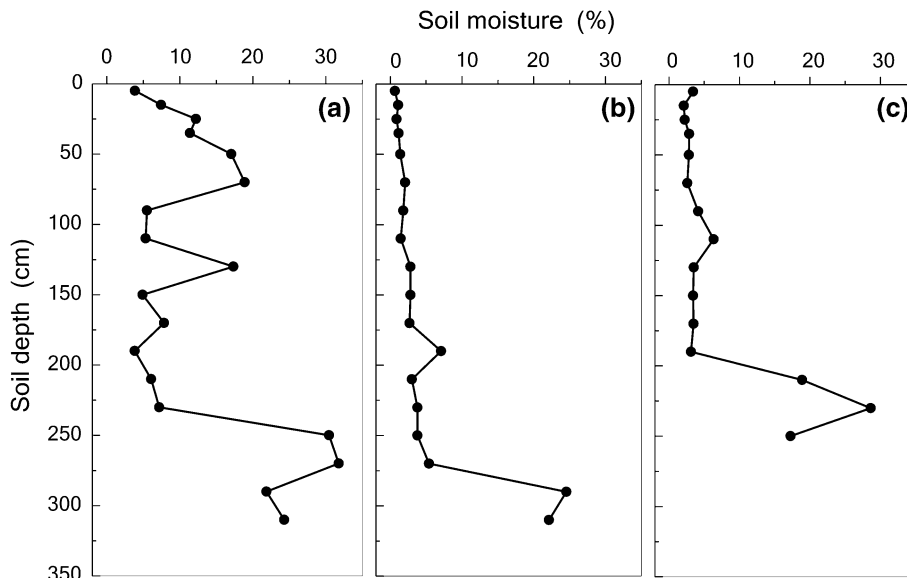


Fig. 2 Soil water profiles in riparian *Populus euphratica* forests patches of different ages along the lower Heihe River; **a** is young forests, **b** is mature forests and **c** is over-mature forests

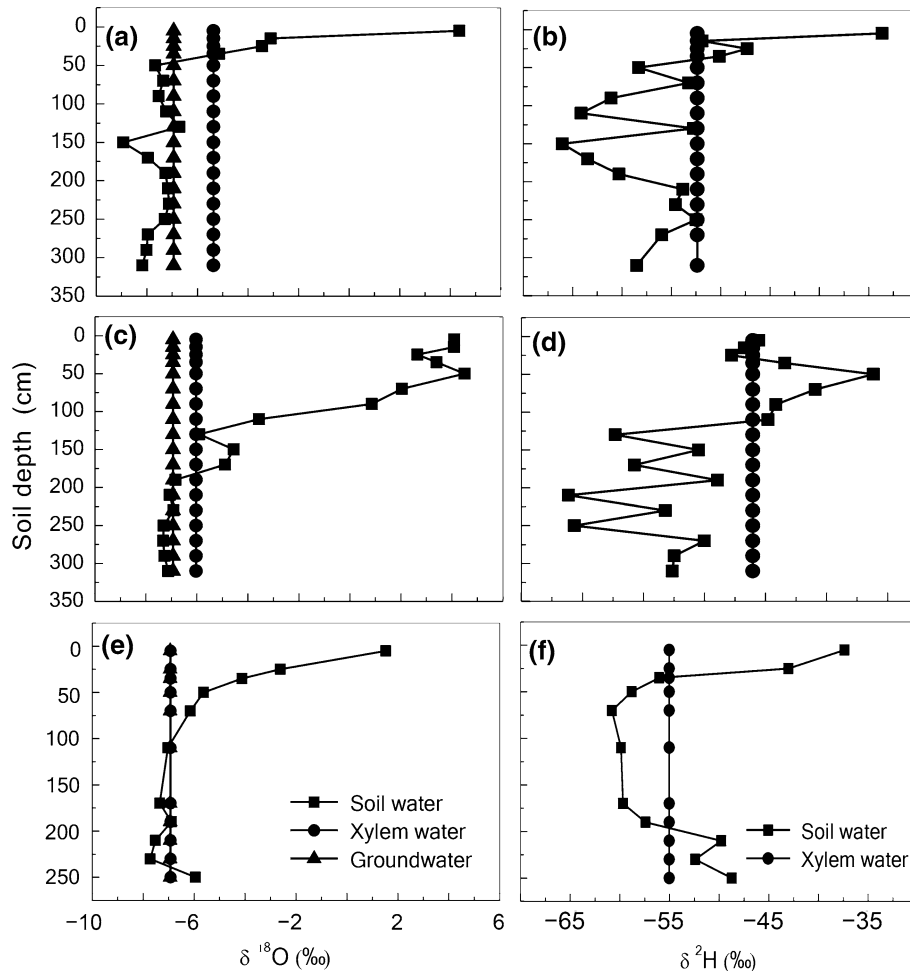


Fig. 3 Comparison of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ profiles of soil water, xylem water and ground water in *Populus euphratica* forests of different age. Figures **a** and **b** are young forests, **c** and **d** are mature forests and **e** and **f** are over-mature forests

xylem water were similar from 100 to 250 (Fig. 3e) cm, suggesting that over-mature forests is also accessing deep water. This interpretation is supported by comparison of $\delta^{18}\text{O}$ values in xylem and groundwater. In young forests, xylem values were considerably higher than those of groundwater (Fig. 3a), suggesting that the trees are using considerable amounts of water from near the ground surface. In mature forests, xylem values were only slightly higher than groundwater values (Fig. 3c), suggesting that trees are primarily using water from deep in the soil, where soil and groundwater $\delta^{18}\text{O}$ values are similar. In over-mature forests, xylem and groundwater $\delta^{18}\text{O}$ values were essentially the same (Fig. 3e), indicating that these trees rely entirely on deep water from near the water table.

The patterns of $\delta^2\text{H}$ in xylem water were most similar to those of $\delta^{18}\text{O}$, but the decrease in $\delta^2\text{H}$ with depth was not as strong as that in $\delta^{18}\text{O}$. In young forests, soil water $\delta^2\text{H}$ decreased from 0 to 150 cm, increased from 150 to 250 cm and then slightly decreased at greater depths (Fig. 3b). In mature forests, $\delta^2\text{H}$ values of soil water increased from 0 to 50 cm, decreased from 50 to 125 cm, and showed no consistent trend at greater depths

(Fig. 3d). In over-mature forests, $\delta^2\text{H}$ decreased from 0 to 70 cm, but then increased from 170 to 200 cm (Fig. 3f). Because of the relatively weak trend of decreasing $\delta^2\text{H}$ with depth, $\delta^2\text{H}$ was less useful than $\delta^{18}\text{O}$ for determining where in the soil profile *P. euphratica* obtained its water.

Modeled contributions by different water sources

We used a mixing model to estimate contributions to xylem water made by different water sources, first considering only $\delta^{18}\text{O}$ and then considering both $\delta^{18}\text{O}$ and $\delta^2\text{H}$. When only $\delta^{18}\text{O}$ was considered, young forests primarily used (40–48 %) soil water from 0 to 50 cm (Table 3), while mature forests used little water from depths of 0–50 cm and 50–100 cm (0–10 % and 0–13 %), and instead derived most of its water from deeper layers and groundwater. Over-mature forests primarily used soil water from 100 to 200 cm, 200 to 260 cm and groundwater (0–92, 0–93 and 0–94 %, respectively), while it used little water from 0 to 50 cm and 50 to 100 cm (0–4 % and 0–18 %, respectively).

Table 3 Proportions of feasible water sources (%) for *Populus euphratica* of different ages (minimum–maximum)

Forest age	Soil depth (cm)	Used $\delta^{18}\text{O}$ and $\delta^2\text{H}$		Only used $\delta^{18}\text{O}$	
		Use ratio (%)	Average value	Use ratio (%)	Average value
Young	0–50	45–48	46.0	40–48	45.8
	50–100	0–39	15.4	0–50	13.4
	100–200	0–23	10.1	0–48	12.9
	200–320	0–43	15.9	0–52	12.9
	Groundwater	0–26	12.6	0–60	15.0
Mature	0–50	1–8	4.5	0–10	3.2
	50–100	0–8	4.2	0–13	4.2
	100–200	1–7	4.0	0–56	17.2
	200–320	0–3	1.3	0–89	38.8
	Groundwater	84–88	86.0	0–91	36.6
Over-mature	0–50	0–3	1.3	0–4	1.2
	50–100	0–16	7.2	0–18	6.8
	100–200	35–54	44.2	0–92	32.9
	200–260	0–49	24.5	0–93	32.1
	Groundwater	0–48	22.8	0–94	27.0

Similar results were observed upon consideration of both the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values (Table 3). Specifically, young forests primarily used (45–48 %) soil water from 0 to 50 cm depth, while it used little from deeper layers. Mature forests used mostly (84–88 %) groundwater, and little soil water from shallower depths. Over-mature forests used mostly (35–54 %) soil water from 100 to 200 cm.

Discussion

All ages of *P. euphratica* along the Heihe River exploit soil layers with the most available water. For all ages of forests, we found a correspondence between the depths of high moisture availability, the depths from which the trees derived most of their water, and the depths with the highest concentration of roots (Brunel et al. 1995). Stable isotopes of oxygen and hydrogen indicated that only young forests derived a large percentage of its water (45–48 %) from near the soil surface (0–50 cm; Table 3). Young forests was also the only age group with significant moisture near the surface (Fig. 2), and the only age group with roots concentrated near the surface (20–40 cm; Table 1). In mature and over-mature forests, stable isotopes indicated that water was primarily derived from deep soil layers and groundwater (Table 3), soil moisture was highest in deeper layers (Fig. 2) and roots were concentrated in deeper layers (Table 1). In contrast, Liu et al. (2012) found that soil water content decreased in layers containing the most absorbing roots of *Caragana intermedia* in Alpine sandland.

Transpiration, evaporation and soil water infiltration could all affect hydrogen and oxygen isotope fractionation in the soil moisture (Gat et al. 1996; DePaolo et al. 2004). Precipitation is low and evaporation is high along the Heihe River, resulting in little downward infiltration of surface moisture to the water table. Except for certain halophytes, plant transpiration has no effect on soil moisture isotopes (Dawson and Ehleringer, 1991;

Mensforth et al. 1994). Therefore, surface evaporation is the main cause of hydrogen and oxygen isotope enrichment in soil moisture along the lower Heihe River. For all ages of forests, soil water $\delta^{18}\text{O}$ values decreased monotonically with depth. High values near the surface reflected strong isotope fractionation resulting from evaporation, while low values were similar to those for groundwater, strongly suggesting that deep soil water is derived from groundwater. These results are consistent with those of previous studies along arid land rivers (e.g., Dawson et al. 1993; Phillips et al. 2001).

Using multiple lines of evidence strengthens investigation of plant water sources. In this study, we combined analysis of stable isotopes of oxygen and hydrogen in xylem water, soil water and groundwater with measurements of soil moisture levels and tree root distribution. The results indicate that the water sources exploited by *P. euphratica* vary with forests age. Young forests uses soil water from 0 to 50 cm, while mature and over-mature forests use soil water from deeper than 100 cm. Water used by young forests is primarily derived from local precipitation, while water used by mature and over-mature forests is derived from groundwater. These results are similar to those reported by Zhao et al. (2008), who found that mature *P. euphratica* in this area use up to 93 % groundwater. These findings are also consistent with water use by trees in other arid and semi-arid desert riparian forests (Scott et al. 2000). In contrast, Zhu et al. (2007) found that both young and mature *P. euphratica* in Ejinaqi exploit deep soil water. This discrepancy could be explained if the young forests of Zhu et al. (2007) was older than the young forests examined here.

The age-related shift in water sources used by *P. euphratica* efficiently exploits the water resource and reduces competition for water between age classes. In some situations, mature and over-mature forests can take up water at depth and release it at shallower levels (Hao et al. 2009), increasing the water available to young forests. Such strategies may increase survival of

this species with a high water requirement in a xeric environment.

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