



Research paper

Variation in soil water uptake and its effect on plant water status in *Juglans regia* L. during dry and wet seasons

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Temporal and spatial variations in the water status of walnut trees (*Juglans regia* L.) and the soil in which they were growing were traced by analyzing the differences in hydrogen isotopes during spring and summer in a 7-year-old walnut stand. Walnut root dynamics were measured in both dry and wet seasons. Walnut roots were mainly distributed in the upper soil (0–30 cm depth), with around 60% of the total root mass in upper soil layers and 40% in deep soil layers (30–80 cm depth). The upper soil layers contributed 68% of the total tree water requirement in the wet season, but only 47% in the dry season. In the wet season, total roots, living roots and new roots were all significantly more abundant than in the dry season. There were significant differences in pre-dawn branch percentage loss of hydraulic conductance (PLC), pre-dawn leaf water potential and transpiration between the dry and wet seasons. Water content in the upper soil layers remarkably influenced xylem water stable-hydrogen isotope (δD) values. Furthermore, there were linear relationships between the xylem water δD value and pre-dawn branch PLC, pre-dawn leaf water potential, transpiration rate and photosynthetic rate. In summary, *J. regia* was compelled to take a larger amount of water from the deep soil layers in the dry season, but this shift could not prevent water stress in the plant. The xylem water δD values could be used as an indicator to investigate the water stress of plants, besides probing profiles of soil water use.

Keyword: branch PLC, gas exchange, *Juglans regia*, root distribution, stable hydrogen isotopes, water source.

Introduction

In the dry season, plants with deep root systems are believed to take water from the deep soil layers, thereby avoiding or minimizing water stress (Sarmiento et al. 1985, Meinzer et al. 1999, Oliveira et al. 2005). However, detailed studies of soil water status, root distribution, water resource derivation and shoot water stress development under natural, varied moisture conditions during the same time period are lacking. Of interest is whether deep water resources can actually compensate for the drought in the air and upper soil layers. *Juglans regia*, which has an extensive root system and wide distribution in the mountainous regions of northern China, is an agriculturally important tree species with high economic value. The eco-physiological responses of the aboveground shoots of *J. regia* in response to drought in their natural habitat, as well as under

controlled greenhouse conditions, have been well studied and the mechanisms underlying these shoot responses are well understood (Picon-Cochard et al. 2001, Cochard et al. 2002, Améglio et al. 2004, Paris et al. 2005, Rosati et al. 2006). However, detailed studies on the role of belowground root structures of *J. regia* during the development of water stress in field environments are lacking. Understanding the role of root processes, as well as the link between roots and shoots with respect to acquisition and utilization of soil water resources, could help clarify the responses exhibited by aboveground shoot water status, as observed in this species during the development and duration of drought.

Roots are difficult to measure, and therefore belowground processes are generally poorly described. In recent years, hydrogen and oxygen isotopic application has contributed

significantly to understanding belowground processes by tracing the processes (Ehleringer and Dawson 1992, West et al. 2006). Hydrogen isotopic mass balance calculations can be used to ascertain water sources under conditions of temporal and spatial heterogeneity in soil water content and isotopic composition. Water taken up and stored in plant roots has the same isotopic composition as the water source (Gonfiantini et al. 1965, Wershaw et al. 1966). During water transport between roots and shoots, the isotopic composition of xylem water remains unaltered from that of the soil (Ehleringer and Dawson 1992). Therefore, it is reasonable to analyze the branch xylem water to determine the water source (Santrucek et al. 2007). The isotopic composition of stable hydrogen is expressed as δD (see Materials and methods for the calculation), which is an expression of the deuterium:hydrogen ratio. The relative quantity of different water sources used by plants can be determined with a two-compartment or three-compartment linear mixing equation (White et al. 1985). Such stable isotope techniques have greatly facilitated the characterization of seasonal patterns of water utilization from different depths in the soil profile (Snyder and Williams 2000, Rose et al. 2003). Isotope signatures thus serve as effective tools to characterize the seasonal dynamics in sources and xylem. Analyses of the natural variation in stable isotopes of hydrogen and oxygen have provided new insights into how ecosystems function across a wide range of spatial scales (West et al. 2006). Those studies mainly include seasonal changes of water resource partitioning with precipitation frequency (Williams and Ehleringer 2000, Sekiya and Yano 2002, Eggemeyer et al. 2009), hydraulic redistribution (Kurz-Besson et al. 2006, Hawkins et al. 2009), and water transport and storage with artificial addition of isotopic tracers (Meinzer et al. 2006, Metzner et al. 2010). With increasing application of isotope techniques, the method would also have a potential use in assessing environmental effects on water balance and water stress in plants. For that purpose, relevant knowledge about relationships between isotope signature and plant water relations is indispensable.

In recent years, an ecosystem restoration program has been implemented in China to improve fragile environments and restore forests in formerly deteriorated ecosystems. This study was conducted in a restored walnut forest located in a rocky hill area of semi-arid northern China. The forest lies in an inland temperate zone, where precipitation varies seasonally due to monsoonal influence. Soil moisture recharge in this region is primarily through rainfall, which mainly occurs in late summer and fall (see Figure 1). Plants frequently experience drought in spring and early summer, which confines most plant growth activities to late summer and early fall. Water is a primary resource that limits terrestrial biological activity, particularly in arid and semi-arid regions (Noy-Meir 1973, Huxman et al. 2004). Soil water is also a key factor in restoring forest

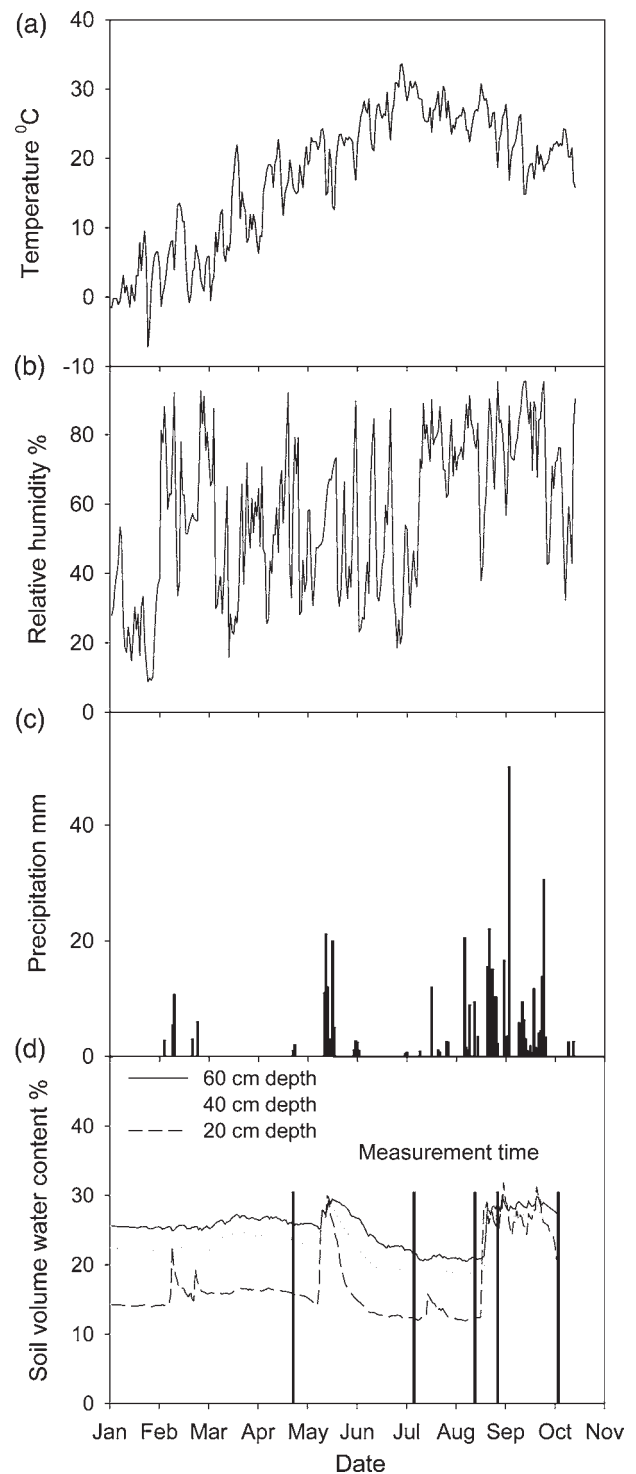


Figure 1. Seasonal changes in mean air temperature, air relative humidity, precipitation events and soil moisture at different depths from January to November in a walnut plantation in northern China. The vertical bars in panel d indicate the sampling and measuring days.

ecosystems in arid and semi-arid zones (Fischer and Turner 1978, Querejeta et al. 2001, Newman et al. 2006), while the efficiency of soil water uptake by trees could be the ultimate determining factor in their productivity. Understanding and predicting ecosystem functioning (e.g., water fluxes) requires an

accurate assessment of plant root distribution (Jackson et al. 1996). Therefore, knowledge of root distribution and mechanisms of soil water extraction and transport by trees is indispensable for successfully restoring ecosystems.

In this paper, we describe observations and measurements of responses of young *J. regia* under contrasting soil moisture conditions. The study had the following main objectives: (i) to detect root distribution characteristics and dynamics; (ii) to analyze patterns of soil water availability at different soil depths in dry and wet seasons using hydrogen isotopes; (iii) to investigate the pattern of root water uptake and its effects on aboveground shoot water status during dry and wet seasons; and (iv) to test if deep soil water uptake can help the plant avoid water stress in drought seasons.

Materials and methods

Experimental sites

This study was conducted at an experimental forest station in Jiyuan, Henan Province (35°01'N, 112°28'E), northern China, from April to October 2009, and included a dry and wet season. The experimental plots were at 310 m altitude, on the south aspect of Tai-hang Mountain, located on the northern border of the Yellow River basin. This region belongs to the inland temperate zone. The mean annual accumulated temperature sum is 5282 °C, and the mean annual precipitation is around 642 mm. Precipitation varies seasonally under the influence of the Asian monsoon. In 2009, the study area experienced very dry conditions from April to mid-August, which led to water stress on plant growth. The wet season started from late August and lasted into early October (for details, see Figure 1). The soil is braunerde weathered from limestone bedrock mixed with aeolian deposits. Gravel makes up 15–28% of the soil at 50–80 cm depth and becomes much denser with depth. Soil pH ranges from 6.8 to 8.5, organic content is around 10 g kg⁻¹, and readily available nitrogen, phosphorus and potassium contents are 2114–8010, 514–616 and 60–103 mg kg⁻¹, respectively (Wang et al. 2007).

Plant materials

This experiment was conducted in a 7-year-old walnut (*J. regia* L.) plantation with 3 m between plants in the rows and 4 m between rows, on level, terraced fields 20 m wide and 140 m long from east to west. The forest was planted to grow walnuts for its nuts and wood, as well as for environmental restoration. Walnut trees there usually start to bear fruits from 5 years of age. The walnut trees had a mean height of 3.4 ± 0.26 m, the south–north extent of crowns was 3.4 ± 0.35 m, and the east–west extent was 3.1 ± 0.49 m. The average diameter of the trunk base was 8.9 ± 0.59 cm, which was determined by measuring every tree. Six walnut trees of similar dimensions were selected for this experiment. The leaves flushed at the

beginning of April and were shed from the middle of October in 2009.

Weather factors and soil humidity measurements

An automatic weather station installed in the walnut forest collected continuous data on air temperature (T_a), relative humidity (H_R) and precipitation (R) during the experimental period. The station was equipped with O5103 sensors (RM Young Inc., Traverse, MI, USA) for measuring air temperature, HMP45C sensors (Campbell Scientific Inc., Logan, UT, USA) for relative humidity and TE525M sensors (Texas Electronics Inc., Dallas, TX, USA) for precipitation, which were connected to a CR10X data logger (Campbell Scientific Inc., Logan, UT, USA). Soil water sensors (Decagon Devices Inc., Pullman, WA, USA) were installed at 20, 40 and 60 cm below the soil surface, 100 cm away from a walnut tree trunk with three replicates. All data on water content were logged every 2 min and the mean was calculated for every 10 min of data logging.

Root distribution and growth status measurement

A minirhizotron root growth measuring system was used to observe dynamic change in roots. Three acrylic minirhizotron tubes (80 cm long and 55 mm in diameter) (Pelican Inc., Torrance, CA, USA) were vertically installed in the soil, 100 cm from each walnut trunk, in April 2008. The inside and outside diameters of the minirhizotron tube were 5 and 5.8 cm, respectively. To allow for root recovery, no observations were recorded in the first year after installation. Root images were recorded three times on 2 July, 27 August and 6 October 2009 using a BTC minirhizotron digital image capture system (Bartz Technology Co., Carpinteria, CA, USA, v4.2), which was introduced into the minirhizotron tube by an aluminum handle, with recording holes at 1.35 cm intervals. Camera depth was controlled by a plastic draw-carriage wheel, which stopped at each recording hole. The measurement image size was 1.8 cm × 1.35 cm. The root images were analyzed with WinRHIZO Tron MF 2005 software (WinRHIZO Pro, Regent Instruments Inc., Quebec City, Quebec, Canada). According to the Hendrick and Pregitzer (1992) method, white roots that are observed in a frame for the first time are considered 'new.' If the roots remain white or brown on subsequent occasions, they are classified as 'living.' Roots are defined as 'dead' when they turn black and produce no new roots on subsequent sampling occasions. The root data were divided into two sections from each minirhizotron tube, one from the 0–30 cm soil layer and the other from the 30–80 cm layer. The separation was mainly based on distribution of root surface area.

In May and October 2009, soil samples, including roots, were excavated and collected at 10 cm intervals from the surface down to 80 cm depth in each sampling site 50, 100, 150 and 200 cm from a walnut stem base by the soil core method (Park et al. 2007). Soil cores (5 cm diameter) were collected near three of the six trees used for the study. If obstructions

were encountered, cores were taken at a slightly different location. After being washed free of soil, the roots were separated into live and dead roots and scanned using the WinRHIZO root scanning system. Root length, surface area, length density and volume were obtained by analyzing the root images with WinRHIZO Reg 2005c software. Afterward, roots were dried at 70 °C to a constant weight and their biomass was quantified. The roots excavated were at least 50 cm from the tree trunk and were thus not very thick (all <6 mm). All roots were pooled together from the four distances (50, 100, 150 and 200 cm) in the same soil layer at 10 cm intervals.

Leaf water potential and branch xylem embolism at pre-dawn

In 2009, measurements of pre-dawn leaf water potential and xylem embolism were taken on 17 April, 2 July, 15 August, 27 August and 6 October. Leaf disks from the fourth or fifth wheel of a fully expanded leaf from a 1-year-old branch were punched. The water potential was measured with a Psypro eight-channel water potential data logger (Wescor Inc., Logan, UT, USA) after at least 30 min of equilibration in a C-52 sample chamber at 25–30 °C. It determines the water potential of samples by measuring the dew point temperature through a thermocouple sensor. The 1-year-old branches were excised at pre-dawn. A segment of each branch was cut for measuring stable isotopes in the xylem water, and the remainder was placed in black plastic bags and carried to the laboratory to measure the percentage loss of hydraulic conductivity (PLC). All measurements on each day were repeated one time for each of the six trees.

Xylem embolism was estimated by PLC from excised branch segments (Sperry et al. 1988). Briefly, a 4-cm-long segment was cut under water with a razor blade, resurfaced with a new razor and attached to a conductivity apparatus (low-pressure flow meter, LPFM) to measure the hydraulic conductivity under gravity-induced pressure gradients. First, a hydrostatic pressure of 5 kPa was used to measure native volume flow rate to calculate initial conductivity (K_i), which was calculated by collecting the efflux continuously with a vial placed on a 0.0001 g balance connected to a computer using LPFM software. The air embolisms were refilled by perfusing the segment at a pressure of 100 kPa with a filtered (0.4 µm) 50 mmol l⁻¹ KCl solution for 10–15 min. A preliminary experiment showed that perfusion for 10 min with 100 kPa was enough to obtain a steady maximal flow rate. Finally, the flow was measured again to calculate maximal conductivity (K_{max}). The percentage loss of hydraulic conductivity was computed as $PLC = 100 \times (1 - K_i / K_{max})$ (Sperry et al. 1988).

Gas exchange

Our preliminary experiment and literature showed that the maximum net photosynthetic rate of *J. regia* occurs around 10:00 h in this region; therefore, leaf gas exchange was

measured in mid-morning with a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE, USA). One-year-old branches were selected and labeled. Red–blue light-emitting diodes maintained incident irradiance at a steady level (1000 µmol m⁻² s⁻¹), and a Peltier cooling module maintained leaf temperature at 25 °C. Five mature leaves between the fifth and sixth wheel from a top branch were chosen for measuring assimilation rate and transpiration. Three measurements were made at each leaf, separated by 15 s intervals. The same procedure was repeated with six trees. The gas exchange measurements were made on the same dates that leaf water potential and xylem embolism were measured.

Tree trunk sap flow

A thermal dissipation probe (TDP) (Dynamax Inc., Houston, TX, USA), with length 20 mm and diameter 2 mm, was used to measure sap flux in the south and north side of the trunk of each *J. regia* tree. Probes were installed at radial depths of 20 mm in the trunk. The height of the installation was 20 cm from the base of the trunk. The sensors were coated with a thermally conductive silicone heat sink compound before being inserted. After being installed, the probes were protected from sunlight with reflective insulation. Signals from the sap flow probes were scanned every 1 min, and 10 min means were recorded by a data logger (CR10X, Campbell Scientific Inc.). This record is synchronized with the weather station. Sap flux is calculated as shown in the following equation (Granier 1987):

$$F_s = 0.0119 \left[\frac{D_{TM} - D_T}{D_T} \right]^{1.231} \times A_s$$

where F_s (l h⁻¹) is sap flux, A_s (cm²) is the sapwood area that was determined by visual assessment of wood disks on the basis of color change from white sapwood to darkish heartwood, D_T is the temperature difference when sap flux is assumed to be zero, and D_{TM} is the maximal temperature difference in one day. Total daily water use (l day⁻¹) was estimated by the sap flow method by summing the total sap flow for each tree, and it was further divided into daytime and overnight water use, which was respectively estimated by the daytime cumulative sap flow from 06:00 to 20:00 h and the overnight cumulative sap flow from 20:00 to 06:00 h.

Stable isotope measurement and contribution rate calculation

Excised segments of branches were immediately sealed in glass vials with polyethylene cone inserts in the cap after removal of the bark. Soil samples under the sampled trees were collected from the upper soil layers (0–30 cm) and deep soil layers (30–80 cm) with the soil core. The samples were all collected from mid-morning to midday of the day. The soils were sealed in glass vials and wrapped with Parafilm® to prevent evaporation.

The vials were stored in a portable cooler (0–5 °C) before being brought to the laboratory and stored at –20 °C. Water was extracted from the branch and soil samples by cryogenic vacuum distillation. Water samples were measured for their hydrogen isotopic composition in an isotope ratio mass spectrometer (Finnigan MAT Delta V advantage, Thermo Finnigan Inc., Austin, TX, USA) interfaced with an elemental analyzer (Flash EA1112 HT, Thermo Finnigan Inc.). The stable-hydrogen isotope (δD) values are expressed relative to Vienna standard mean ocean water (V-SMOW) in ‰, as shown in the following equation:

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

where R refers to the hydrogen stable isotopic composition (D/H ratio) of a sample and standard, respectively. Hydrogen isotopic composition of the upper soil varied greatly due to evaporation. Therefore, the δD in the upper soil layers was a weighted average of the measured δD of soils sampled at 5, 15 and 25 cm depth. The δD value was weighted by gravimetric water content (W_g) at each depth by dividing mean W_g at each depth by the sum of W_g at all depths. The δD of deep soil was measured with a mixed soil sample from 30 to 70 cm. The ground water was not measured because it was impractical to obtain soil samples from deeper than 80 cm due to the underlying rock at most sites. Gravel diameter was relatively small (around 3–5 cm diameter) and the gravel layer was fairly homogeneous. When the δD of xylem water differed from the δD of soil water from the different layers, the higher δD source was considered as the enrichment end and the lower as the dilution end. The total plant xylem water derived from the upper soil layers (0–30 cm) and the deep soil layers (30–80 cm) was calculated using a two-compartment linear mixing equation (White et al. 1985):

$$\delta D_t = x_1 \delta D_s + x_2 \delta D_d$$

$$x_1 + x_2 = 1$$

where δD_t is the measured δD of tree xylem sap, δD_s and δD_d refer to the δD values of upper soils and deep soils, respectively; and x_1 and x_2 denote the contribution rate of total plant water obtained from the upper soil layer and the deep soil layer, respectively, assuming no other water source was available for trees in this system.

Statistical analysis

Analysis of variance and multiple comparisons (least significant difference) between means of water relations and gas exchanges over time, and of root dimension in different depths and over time were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Pearson's correlations between δD of branch water and

water relations, and gas exchanges, and between daily sap flow and transpiration, and air temperature were analyzed using linear functions. The significance levels were set at $\alpha = 0.05$.

Results

Weather factors and soil humidity

The annual precipitation in 2009 was 416 mm, with only 132 mm falling from January to July, during which dry conditions resulted from a combination of high temperature and limited precipitation (Figure 1). Rain events mainly occurred in August and September. From 16 August on, rain events rapidly increased and precipitation summed to 224 mm by 6 October. Water content at 20 cm soil depth was readily changed from 12 to 32% due to evapotranspiration and rain events. Soil water content at 40 cm depth changed from 18 to 30%, and that at 60 cm depth changed from 20 to 30%. Soil water contents at field capacity and wilting point were 34.4–37.2% and 10–11%, respectively (He and Han 2006).

Root biomass

Walnut root growth differed significantly between the dry season and wet season (Figure 2). Mean root length in both the upper (0–30 cm) and deep (30–80 cm) soil layers was shortest on 2 July, when the soil water content and air relative humidity were lower (Figure 1a and d). After rain events, the total root length on 27 August and 9 October increased ($P < 0.05$) by 128% and 179%, respectively, compared with that on 2 July (Figure 2a and b). The abundance of new roots significantly increased in both the upper and deep soil layers in response to the rain events. The growth of new roots was greater in the upper soil profile than in the deep soil profile. Dead root length in the upper soil layer was significantly higher in the wet season than in the dry season, while no difference in dead roots was detected in the deep soil layer between the seasons. The diameter of the roots did not significantly change by season.

There was significant variation in the vertical distribution of roots (Table 1). Roots were the most abundant at 10–30 cm depth, followed by 0–10 cm depth. Root biomass decreased with depth below 30 cm. Generally, most of the root surface area, root length density and root biomass were confined to the upper soil layers (0–30 cm), accounting for 60.9, 62.2 and 78.9% of the total root measurements from the 0–80 cm soil layers, respectively.

Dry and wet season δD values of water in the soil and plant branches

The δD values of upper soil water differed significantly between seasons. The δD values increased with soil drying, but sharply dropped to those of rainwater after rain events (Figure 3a). In contrast, δD values in the deep soil water were nearly constant

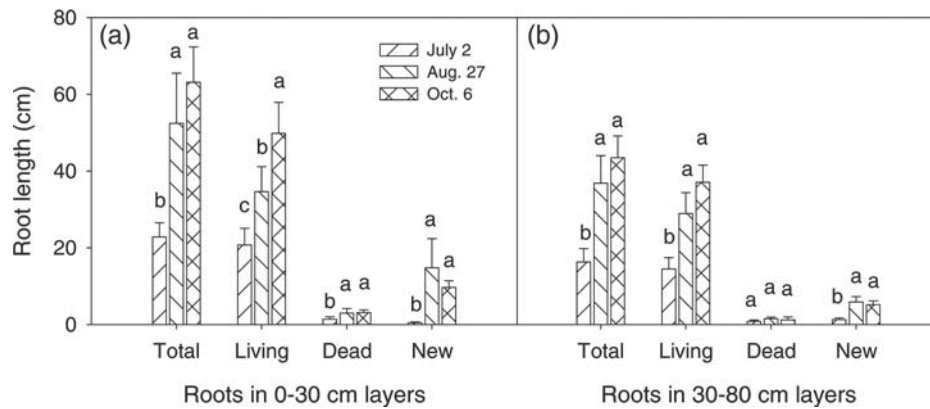


Figure 2. Dynamics of walnut root length (a, b) at different soil layers, detected by the minirhizotron system, over the growing season. Means and standard deviation (SD) are shown ($n = 3$). Different letters above the bars refer to significant difference at the $P = 0.05$ level.

Table 1. Vertical root distribution in different soil layers. Roots from four different distances (50, 100, 150 and 200 cm) from the tree trunk in the same soil layer from each sampling location were pooled. Three sampling locations were used. Means and SD are shown ($n = 3$). Different letters refer to significant difference at the $P = 0.05$ level within the same sampling time.

Time	Depth (cm)	Surface area (cm ²)	Average diameter (mm)	Root length density (cm/dm ³)	Weight (g)
May	0–10	65.4 ± 19.4 b	0.8 ± 0.2 b	466.3 ± 52.6 b	1.3 ± 0.4 ab
	10–20	103.0 ± 17.9 a	1.0 ± 0.2 a	541.7 ± 93.6 a	2.3 ± 1.1 a
	20–30	121.3 ± 11.6 a	1.0 ± 0.2 a	257.3 ± 26.5 c	1.8 ± 0.5 ab
	30–40	61.1 ± 16.5 b	0.8 ± 0.2 ab	201.9 ± 61.2 cd	1.2 ± 0.6 b
	40–50	54.0 ± 14.5 b	0.8 ± 0.1 b	179.0 ± 61.4 cd	0.8 ± 0.3 bc
	50–60	39.9 ± 3.8 c	0.7 ± 0.1 b	152.6 ± 24.8 d	0.6 ± 0.1 c
	60–70	23.7 ± 3.3 d	0.6 ± 0.1 bc	112.7 ± 27.4 de	0.3 ± 0.1 d
	70–80	13.5 ± 1.8 e	0.4 ± 0.1 c	81.0 ± 18.0 e	0.2 ± 0.1 d
October	0–10	82.1 ± 32.6 b	0.7 ± 0.2 c	539.9 ± 78.6 b	2.3 ± 0.7 ab
	10–20	142.6 ± 53.3 a	0.8 ± 0.2 bc	720.7 ± 82.5 a	2.9 ± 0.7 a
	20–30	131.8 ± 35.1 a	1.3 ± 0.4 a	408.7 ± 52.9 c	3.6 ± 1.3 a
	30–40	76.1 ± 20.4 b	1.0 ± 0.4 ab	344.9 ± 39.5 cd	2.0 ± 1.1 ab
	40–50	60.7 ± 15.9 bc	1.0 ± 0.3 b	230.2 ± 25.4 e	1.3 ± 0.7 b
	50–60	40.5 ± 12.0 c	0.8 ± 0.2 bc	190.2 ± 31.4 e	0.7 ± 0.5 bc
	60–70	37.5 ± 13.5 cd	0.9 ± 0.2 bc	171.3 ± 22.1 e	0.5 ± 0.3 c
	70–80	25.0 ± 9.3 d	0.8 ± 0.2 c	120.5 ± 20.9 f	0.2 ± 0.2 d

over the experimental period. The δD values of xylem water in *J. regia* branches were significantly higher in the dry season than in the wet season. During the dry season, more than half of the xylem water came from the deep soil, while in the wet season, the xylem water was mainly extracted from the 0–30 cm soil layers (Figure 3b). Correlation analysis showed a significant correlation between the δD values of xylem water and the water content in shallow soil layers (both at 0–20 and 20–40 cm depths), but a not so significant correlation with those in the deeper soil layers (40–60 cm depths) (Table 2).

Roots took up water with different efficiencies between the dry and wet seasons. The water extracted from the upper soil (0–30 cm), where the root surface area accounted for ~60% of the total roots, contributed only 47% of the whole tree water requirement in the dry season. In contrast, the root surface

area in the 30–80 cm layer accounted for 40% of the total roots but absorbed up to 53% of the water total. In contrast, the water absorbed by upper roots accounted for 68% of the total during the wet season, while that absorbed by deep roots accounted for only 32% (Figure 4).

Plant–water relations

There was a significant difference in pre-dawn embolism between the dry and wet seasons. The pre-dawn embolism of branches was 23.20% and 26.60% on 2 July and 15 August, respectively, and higher than the values of 17.56% and 16.25% on 27 August and 6 October, respectively (Figure 5a). Pre-dawn leaf water potential varied by season. As the drought progressed, the water potential reached a minimum of -1.51 MPa on 15 August. After rain events, the water potential

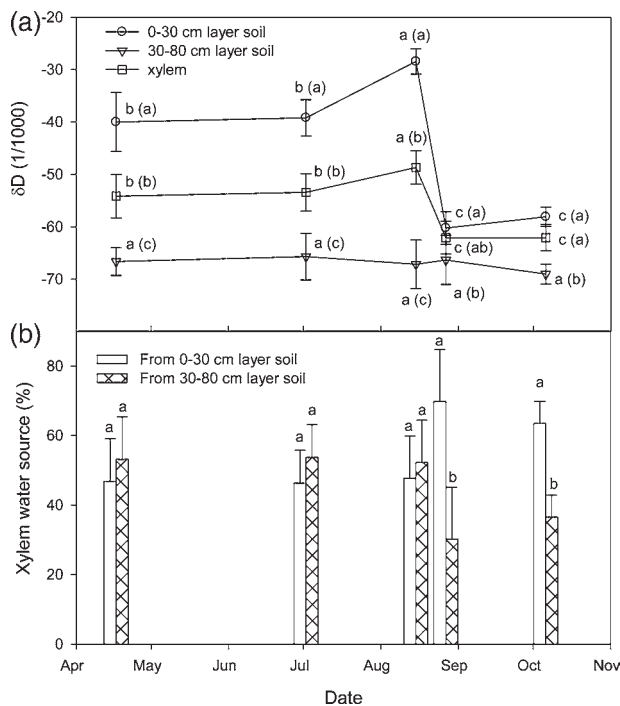


Figure 3. Changes in deuterium isotope content in xylem water and soil (a) and percentage of water source in the xylem (b). Means and SD are shown in panel a ($n = 6$). Different letters refer to significant difference in δD in the same soil layer or xylem water, and different letters in parentheses refer to significant difference in δD of soil and xylem water sampled on the same date at the $P = 0.05$ level.

Table 2. Relationship between the volumetric water content of the soil and the δD values of the xylem water in different soil layers.

	Regression equation	R value	R^2 value	P value
0–20 cm depth	$Y = -0.360x - 5.729$	0.73	0.53	0.002
20–40 cm depth	$Y = -0.254x + 1.790$	0.55	0.30	0.033
40–60 cm depth	$Y = -0.171x + 8.891$	0.51	0.26	0.054

rapidly increased and was significantly higher than that in the dry season (Figure 5b).

The leaf transpiration rate exhibited similar dynamics to the pre-dawn water potential in the growing season (Figure 5c). There was a significant difference in transpiration between the dry and wet seasons. During the dry season, the transpiration rates ranged as $0.9\text{--}1.6 \text{ mmol m}^{-2} \text{ s}^{-1}$, significantly lower than the range of $2.1\text{--}2.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ observed in the wet season. The assimilation rate did not completely follow the dynamic pattern of transpiration (Figure 5d). Photosynthetic rate was lowest on 15 August, when the soil moisture was lowest in the growing season (Figure 1d); however, the highest photosynthetic rate occurred on 17 April, when the soil moisture was not highest.

The δD of xylem water was closely correlated to the branch PLC measured at pre-dawn ($P < 0.001$) (Figure 6a). The embolism level increased with xylem δD . Similar analyses were performed between xylem δD and leaf pre-dawn water potential, leaf transpiration and photosynthesis. The former two

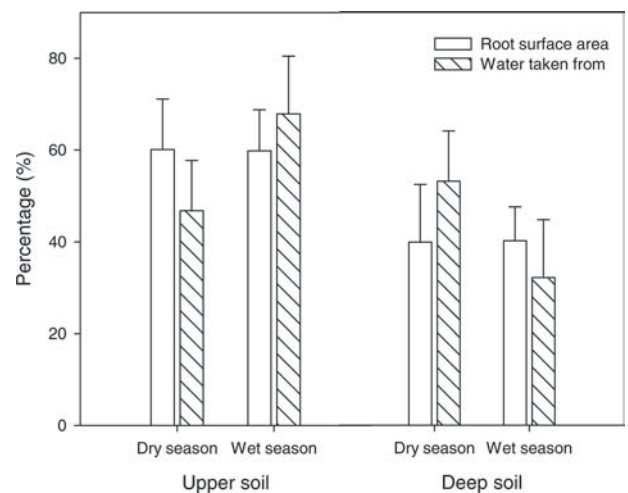


Figure 4. Root distribution and water uptake from the upper and deep soil layers in different seasons. Upper soil refers to the first 30 cm, and deep soil refers to the 30–80 cm layers. The error bars refer to SD.

parameters had significantly negative and linear correlations with xylem δD , while photosynthesis was not significantly correlated with xylem δD (Figure 6b–d).

The daily sap flow varied significantly between the seasons and was mainly determined by the daytime sap flow (Figure 7). Generally, in summer, even in early October, the flow was higher than in spring (17 April). On 15 August due to the lowest soil moisture, the daily sap flow was also restricted. The daily sap flow was significantly correlated to transpiration demand and also to mean air temperature (Table 3). For all individuals, the sensors showed negligible night-time sap flow with lowest values on 15 August.

Discussion

Changes in soil moisture and δD

As shown in Figure 1c and d, while evapotranspiration gradually consumed soil water from April to mid-August, rainfall, especially heavy rainfall events, rapidly increased the pool sizes of soil water in late summer. This study showed that the δD value of water in the upper soil gradually increased from April to August with the progression of soil drought, while the δD value of water in the deep soil was relatively stable. The isotopic composition of soil water varied with depth because water near the soil surface became enriched in heavier isotopes as a result of evaporative fractionation (Allison and Leaney 1982). Additionally, hydraulic redistribution can be a factor that may influence isotopic signature in the upper soil layer (Dawson 1993). However, the contribution would be relatively small or constant in our experiment. In the dry season the δD value of water in the upper soil gradually increased, and in the wet season rainfall dominated the effect of isotope composition on the upper soil layer (Figure 3). Rain events sharply

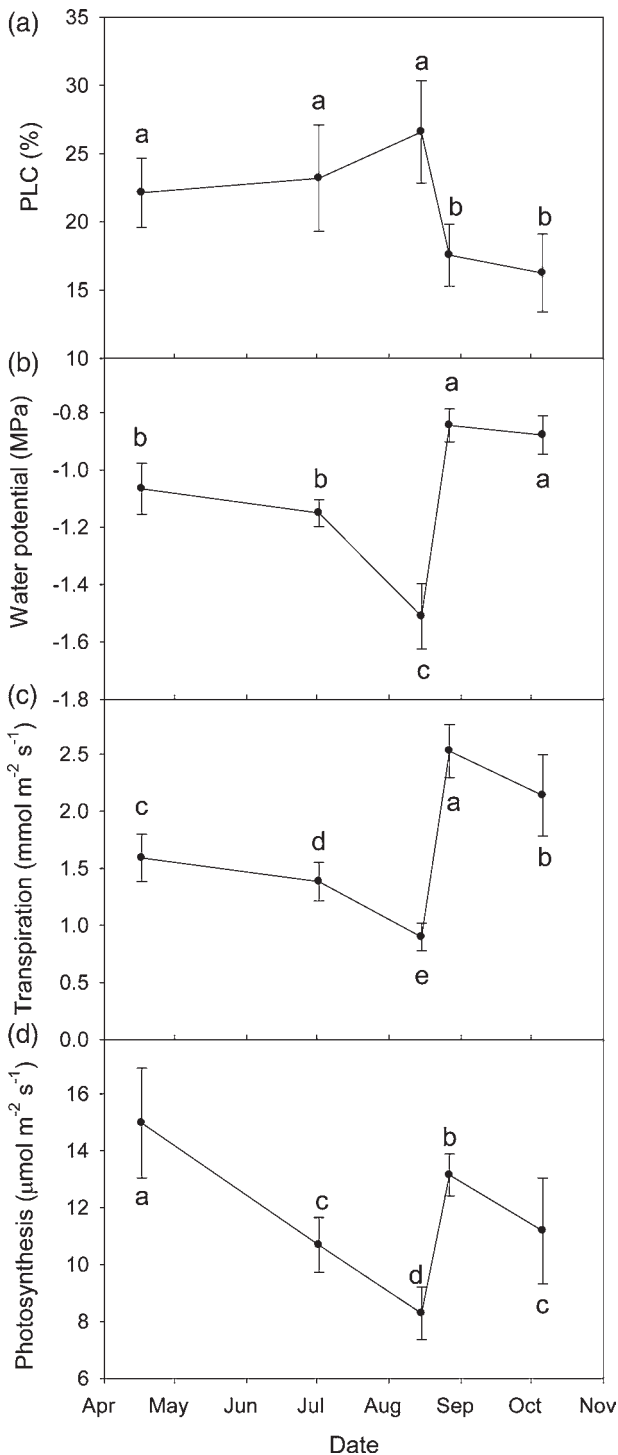


Figure 5. Variations in (a) pre-dawn branch xylem PLC, (b) pre-dawn leaf water potential, (c) leaf transpiration and (d) assimilation over the growing season. Means and SD are shown ($n = 6$). Different letters above the bars refer to significant difference at the $P = 0.05$ level.

changed δD to that of rainwater in the upper soil layers. The δD value of rain varied from -69 to -61‰ during the 2009 wet season, in a range consistent with a previous report (Yano et al. 2006).

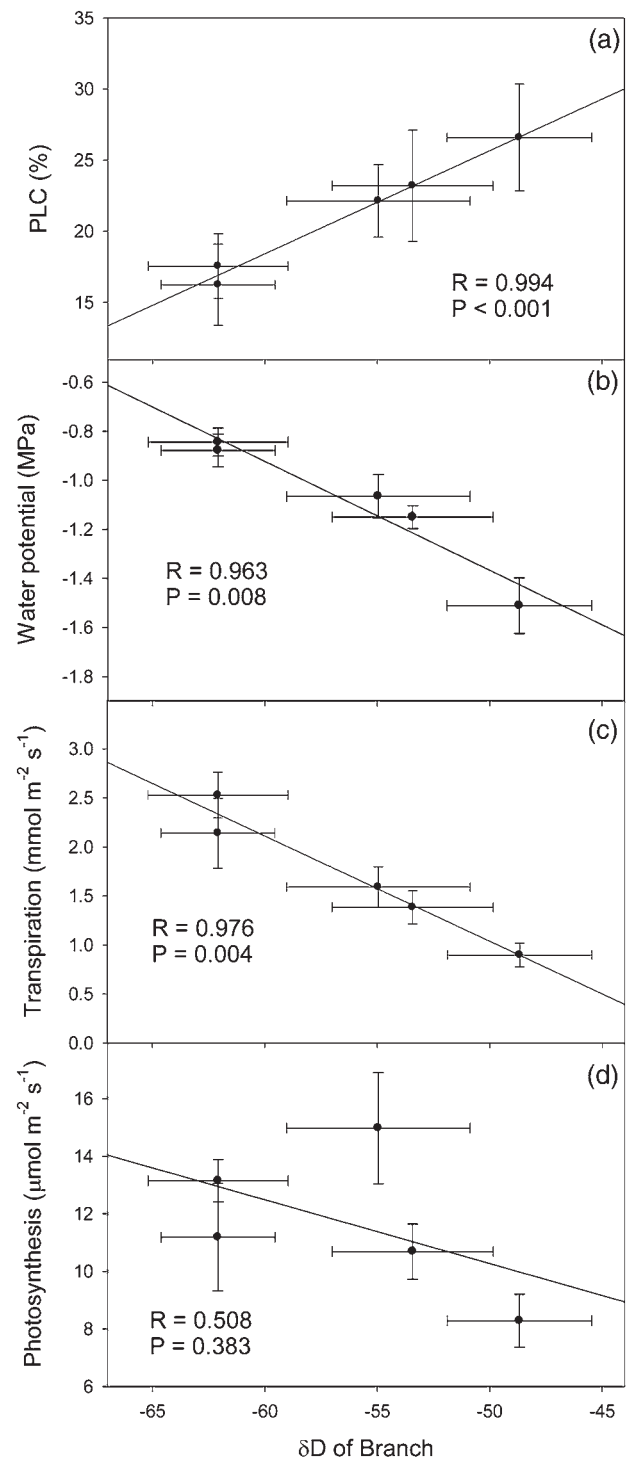


Figure 6. (a) Pre-dawn branch xylem PLC, (b) pre-dawn leaf water potential, (c) transpiration and (d) assimilation as a function of the δD values of branch xylem water. Linear Pearson's correlation was performed between these measurements. The error bars refer to SD.

Changes in plant roots

Roots are difficult to study because they are underground and hidden from view. Root distributions also often vary within the soil. Therefore, in this study, we employed two methods to

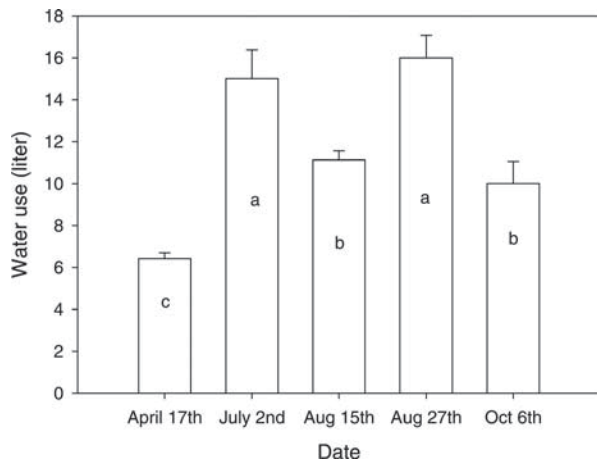


Figure 7. Mean water use (l) estimated by the sap flow on three individuals. Data of each tree were derived from two TDP probes, respectively installed in the south and north side of the trunk. Means and standard deviation (SD) are shown ($n = 3$). Different letters refer to significant difference at the $P = 0.05$ level.

Table 3. Relationships between the daily sap flow and transpiration and air temperature.

	Regression equation	R value	R ² value	P value
Transpiration	$Y = -1.770 + 0.398x$	0.951	0.904	0.013
Air temperature	$Y = -2.141 + 0.541x$	0.894	0.800	0.041

detect root distribution and dynamics during the growing season. One was the minirhizotron technique, which is a modern, non-destructive method adapted to study root dynamics in the natural soil environment. In parallel, a traditional method of soil coring was used with the WinRHIZO root scanning system. Data collected by the two methods agreed well with each other (Table 1, Figure 2), and showed that *J. regia* had a deep root distribution (but more roots (60%) were found in the upper soil layers) and that rain events promoted root growth after drought. This root distribution is consistent with a global database showing higher fine root densities in the upper soil layer (Jackson et al. 1997). Fine root growth was previously found to be closely correlated with change in soil water content in the study area (Zhang et al. 2002). The root distribution pattern remained unaltered between the two moisture-contrasting seasons, although total roots, living roots and new roots in the wet season were all significantly more abundant than in the dry season (Table 1, Figure 2). Drought in the upper soil could inhibit root growth in the dry season; however, moisture in the deep soil layers should not be the restriction factor deterring root growth. The fact that differential moisture contents in the different soil layers did not cause variation in the root distribution pattern suggested that the restricting factor should be from other processes instead of the soil moisture. The reduced assimilation during the drying period could limit the carbohydrate supplement which might be the factor restricting root growth.

The root distribution pattern did not change between the dry and wet seasons, but the water uptake efficiency was significantly different between these seasons (Figure 4). During the dry season, the soil water potential in the shallow layers might be too low for roots to efficiently take up water from these layers. Root hydraulic conductivity has been found to be inhibited by soil drying (Lo Gullo et al. 1998, Vandeleur et al. 2009). Additionally, hydraulic conductivity could be reduced in areas where cracks appear between the soil and roots during soil drying. Therefore, a greater ratio of water was taken from the deep soil than from the upper soil in the dry season. In contrast, rainfall sharply increased the soil water content and hence the water potential. When the soil water content increased to a certain threshold, the upper roots could establish and maintain the function of absorbing soil water (Dawson and Pate 1996). Soil moisture was closely related to new root growth (Figure 2, Table 1). In September and October, when the soil temperature and phenological stage were not the limiting conditions for root growth, soil moisture was a key factor in regulating root growth. New roots are especially efficient in water uptake because of high hydraulic conductivity (Wan et al. 1999).

Changes in hydraulic characteristics and δD of plant aboveground parts

As shown in Figure 1c and d, while evapotranspiration gradually consumed soil water, rainfall, especially heavy rainfall events, rapidly increased the pool sizes of soil water. Changes in the pool sizes in turn affected plant physiological activities related to water relations, such as root growth, stem hydraulic conductance, leaf transpiration, photosynthesis and stem sap flow (Figures 2, 5 and 7). With the pool sizes shrunk, the bulk hydraulic resistance of trees increased, reflected in an increase in PLC and a decrease in water uptake by upper roots. The increase in hydraulic resistance in turn resulted in the rapid decline of leaf transpiration, consistent with the report of Cochard et al. (2002) on this species. At the same time, the capacitance of trees decreased, manifested with a decrease in pre-dawn water potential. Intense precipitation, in contrast, rapidly restored the physiological processes. Water potential and transpiration even recovered to the highest values of the whole season, whereas water use on August 27 was in the same order of magnitude as on July 2. These physiological responses are consistent with the report of Phillips et al. (2004) on the time series diagnosis of tree hydraulic characteristics. That is, these results fit well to a first-order transient response model of hydraulic resistance and capacitance within trees after a rain disturbance.

As soil dried in the upper soil layers, xylem tension increased. Regression analyses showed that the δD values of xylem water were correlated with pre-dawn branch PLC, pre-dawn leaf water potential and transpiration (Figure 6). However, unlike

transpiration, assimilation rates were not significantly correlated with the δD values of xylem water, suggesting that some non-stomatal factors played a role in restricting the photosynthesis in walnut trees (Rosati et al. 2006). Branch xylem embolism (PLC) is associated with a decreased transportation efficiency of water and with plant water stress status (Sperry et al. 1988). Moreover, embolism, or cavitation, in the stem as water deficits increase can act as a hydraulic signal, substantially lowering leaf water potential (Jones and Sutherland 1991, Sperry et al. 1998) and stomatal conductance (Fuchs and Livingston 1996). Pre-dawn leaf water potential is thought to equilibrate closely with soil/root water potential, and thus is often used as an indicator of soil or root water potential to avoid some of the problems associated with direct soil measurement (Sala et al. 1981, Ryel et al. 2003). Changes in pre-dawn leaf water potential indicated that the soil experienced progressive drought from April to mid-August, which was also reflected in the dynamics of δD in xylem water.

In 2009, the study region experienced very dry conditions, and the annual precipitation was 416 mm, ~226 mm less than the previous long-term average. In response to water stress, walnut trees regulated their transpiration by decreasing their stomatal conductance to avoid dehydrative damage to their cells and tissues. As a result, this reduced their photosynthetic potential. During the peak of drought, on August 15, photosynthesis was 37% lower than on August 27 after the first rainfall. The drought greatly reduced tree production. Carbon starvation may be particularly likely if drought lasts longer than the equivalent amount of plant carbon reserves (McDowell et al. 2008).

Relationship between variation in water source partitioning and plant water status

Comparison of the δD values in plant stem water and soil water at different depths demonstrated that *J. regia* was compelled to take a higher ratio of water from the deep soil layers in the dry season. However, measurements of water relationships indicated that the larger water uptake from deep soil was not able to deter water stress on the plants (Figure 3 and 5). Deep soil water resources may allow plants with deep root systems to survive in dry seasons (Brooks et al. 2002, Meinzer et al. 2004, Domec et al. 2006). Also, deep soil water supplementation could maintain the hydraulic conductivity of roots in the nutrient-rich upper soil throughout the dry season (Lo Gullo et al. 1998), keeping roots ready to extract water when moisture becomes available in the upper soil. Otieno et al. (2006) found that *Quercus suber*, with a deep root system, took up most of its required water from the deep soil layers during drought to maintain good water status, but no growth was recorded during this time. Water in the upper soil layers, however, seemed to play a more important role in tree productivity. Values of δD trace the ratio of water sources, but not the

absolute amount of water. Lower δD values suggest that xylem water has a higher ratio of water from the deep soil layers, but cannot be automatically translated into greater water uptake from the deep soil layers. Such a finding could also indicate reduced water uptake from the upper soil layers or a mixture of reduced water uptake from the upper layers and increased water uptake from the deep layers. Thermal dissipation probe transpiration measurements indicated that the daily sap flow decreased by around 30% on the driest day in comparison with 2 July and 27 August (Figure 7), suggesting that the highest xylem δD on 15 August would be mainly attributed to reduced water uptake from the upper soil layers. Additionally, the δD values in xylem water were significantly correlated with the shallow soil layers (0–20 and 20–40 cm depths), but not so significantly correlated with the deeper soil layers (40–60 cm depths) (Table 2), suggesting that water uptake by walnut would tend to be mainly determined by water supply of the upper soil. During water transport between roots and shoots, the isotopic composition of water remains unaltered; therefore, it is reasonable to believe that water in sap flow was also mainly provided by the upper soil. Many studies with stable isotopic hydrogen and oxygen on seasonal changes in water sources investigated the water source shift from upper to deep soil layers with decreasing precipitation, and the results sometimes imply that water uptake from deep soil, where water is available, could solve the drought problem. Without a doubt, the extraction of water from deep soil layers could alleviate drought stress on plants; however, the shift cannot always maintain water balance in plants and avoid drought stress. One should, therefore, be cautious when interpreting the results.

In this study, comparison of the δD values of plant stem water and soil water at different depths revealed the existence of different water source partitioning patterns between different soil moisture conditions in a planted walnut forest in northern China. The δD values showed that plants mainly used water from the upper soil in the wet season, while upper and deep soil water more or less equally contributed to plant xylem water in the dry season. The result is consistent with that of previous studies. McCole and Stern (2007) reported a change in juniper water use from a predominantly deep water source during summer, when it was hot and dry, to a predominantly upper soil source during winter, when it was cool and wet. *Pinus edulis* and *Juniperus osteosperma* largely use monsoon precipitation during the monsoon period, but use of this precipitation declines sharply with decreasing summer rain input (Williams and Ehleringer 2000). In this paper, we assume that no other water source was available for trees in this system. However, the roots might penetrate through the dense gravel layers and may be in contact with groundwater. Therefore, the influence of groundwater on xylem isotopic signature cannot be completely excluded, although Williams and Ehleringer (2000) found that

plants did not use groundwater in the pinyon–juniper ecosystem of the southwestern USA, a site similar to this study region. Nevertheless, it should be noted that the seasonal change of water resource partitioning was based on the two measured depths.

Conclusions

Walnut roots were mainly distributed in the upper soil layers at our study site and likely in the whole region. Soil moisture was a key factor regulating root growth and water uptake efficiency of the roots. The shallow roots had reduced efficiency in water uptake in the dry season, and therefore *J. regia* was compelled to extract a greater ratio of water from the deep soil layers. However, the shift was not able to prevent water stress on the plants, which was characterized by increased pre-dawn branch xylem PLC, reduced pre-dawn leaf water potential and transpiration with soil drying. In addition to serving as an indicator of water sources, changes in the δD values in walnut branch xylem water reflected plant water status and the severity of soil drought.

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