



Seasonal effects of intercropping on tree water use strategies in semiarid plantations: Evidence from natural and labelling stable isotopes

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Abstract

Aims Intercropping in plantations can improve ecosystem services, but its potential effects on trees' water use and production are concerns due to increases in water scarcity related to climate change. The aim of this study was to address these concerns by exploring water uptake responses of jujube (*Ziziphus jujuba* Mill.) trees to intercropping and extremely dry periods on the semi-arid Loess Plateau of China.

Methods Natural stable isotopes (^2H and ^{18}O) were analysed to characterize water use patterns of jujube trees and intercrops in the main tree and crop root overlap layer (ROL, 0–120 cm). 10% stable deuterated water was injected at three targeted depths (2, 3 and 4 m) to characterize the water uptake of jujube trees below the main root overlap layers (BOL).

Results In ROL, intercropped jujube trees obtained higher proportions of water in shallower and deeper

layers than monocultured jujube trees during wet and dry periods, respectively. Proportional contributions of soil layers to the trees' water uptake were positively correlated with the layers' water amount ratios (relative to the entire profile) in intercropped orchards but not in monoculture. In some extremely dry periods, intercropping resulted in jujube trees absorbing deeper water (up to 3 m) in BOL.

Conclusions At the early stage of land-use change from plantation to agroforestry, intercrops induce jujube trees to absorb higher proportions of water from soil layers with high proportions of total water contents in ROL. The soil water in BOL is an important buffer for maintaining water supplies for tree growth in agroforestry systems in case of extreme drought.

Keywords Agroforestry systems · Natural and stable-isotope-labelling techniques · Water uptake patterns · Extreme drought

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Introduction

One of the main strategies for addressing the problems and enhancing the ecosystem services of degraded landscapes is to establish plantations (Feng et al. 2005; Payn et al. 2015). However, the effectiveness of tree monocultures is often compromised by their simple structures and associated limitations of ecological functions, such as carbon sequestration, soil conservation, water retention and ecological stability (Jia et al. 2012; Ling et al. 2017; Vesterdal et al. 2008). Agroforestry, an ecosystem

with multi-layered structure that combines forestry and agricultural techniques, is a promising solution that is expected to buffer the microclimate, improve soil structure and increase biodiversity (Chen et al. 2019; Jose 2009; Lin 2010). However, in water-limited regions, planting intercrops significantly changes hydrological processes and the ecosystems' water balance (Fernández et al. 2006; Gao et al. 2013; Sanchez 1995). Therefore, it is important to study the effects of the presence of crops on trees' water use.

Accumulated evidence has demonstrated that intercropping can enhance soil's water holding capacity and reduce its evaporation rates by increasing soil organic matter contents, enhancing the formation of stable soil aggregates and regulating the microclimate (Chen et al. 2019; Schwab et al. 2015; Wu et al. 2016), thereby improving soil water availability. However, due to anticipated climate change, more intense and frequent droughts are expected, particularly in semiarid regions (Huang et al. 2017; Porporato et al. 2004), which may lead to interspecific water competition and offset the potential benefits mentioned above (Fernández et al. 2006; Gao et al. 2013). Some studies have found that extreme drought reduced water availability and the productivity of agroforestry systems (Fernández et al. 2006; Ling et al. 2017; Payn et al. 2015). In such cases, the use of trees with deep and dimorphic roots that can flexibly adjust their water sources according to changes in the soil water profile may be essential for maintaining ecosystems' stability and productivity (Canadell et al. 1996; Fernández et al. 2008; Yang et al. 2015).

As soil water content (SWC) is one of the most important factors affecting plants' water use strategies (Gao et al. 2018; Nnyamah and Black 1997), the relationship between its distribution and contribution to plants' water uptake has received much attention. For example, Green and Clothier (1995) and Green et al. (1997) found that both kiwifruit vines and apple trees can quickly adapt following partial wetting of the root zone and begin taking up substantial amounts of water from the wetted layers. In addition, Liu et al. (2011b) found that water use patterns of two species of shrubs in western China significantly correlated with the soil water profiles when the soil water in shallow layers was sufficient. Besides, Gao et al. (2018) found that the contribution of shallow soil water was significantly and positively correlated with the soil water content when they studied water use patterns of two species of shrubs in China's Loess Plateau. These studies all

indicated that the soil water profile determines water use patterns of plants to a certain extent, but they focused on monocultures and did not address the effects of commensal species. Therefore, to better understand trees' responses to intercropping, it is important to investigate intercrops' effects on soil water profiles, trees' water uptake patterns and the relationships between them.

The analysis of natural stable isotopes of hydrogen and oxygen (δD and $\delta^{18}O$) of water provides a reliable and efficient method to measure the water uptake patterns of a given plant (Dawson and Pate 1996; Ehleringer and Dawson 1992). The main advantage of this approach is that proportional contributions of different water sources can be quantitatively differentiated. Studies using this method in agroforestry systems have mostly focused on water competition between species (Eggemeyer et al. 2009; Fernández et al. 2008; Wu et al. 2017). Therefore, researchers have largely addressed the water cycle in the main crop/tree Root Overlap soil Layers (ROL), from which both crop and tree roots acquire water resources (Wu et al. 2016, 2017). However, in tree-based agroforestry systems, the presence of intercrops may induce deeper rooting of trees (Cardinael et al. 2015; Celette et al. 2008), and affect water uptake patterns of trees not only in the ROL, but also below the main root Overlap Layers (BOL, Fig. 1), which may be crucial for their tolerance of extreme drought (Grossiord et al. 2017). Despite the importance of this hydrological partitioning, there is little direct evidence regarding the spatio-temporal patterns of trees' water uptake from BOL in agroecosystems. This is mainly because there is no clear vertical gradient of natural soil water isotope composition in deeper layers, so its analysis is no longer informative (Ehleringer and Dawson 1992). Fortunately, artificial labelling, using a stable isotope of water (deuterium, 2H) as a tracer, has proven utility for studying water uptake dynamics of plants in deep soil layers (Beyer et al. 2016; Beyer et al. 2018; Grossiord et al. 2014). Therefore, combining analyses of natural and artificial isotopic signatures may be ideal for studying the water uptake dynamics of agroforestry systems in both ROL and BOL.

China's Loess Plateau has both the world's largest deposits of loess and some of the most severe erosion and associated problems globally (Liu 1999). To address these problems and increase farmers' income, jujube (*Ziziphus jujuba* Mill.), a perennial fruit tree with deep roots (Jiang et al. 2007; Li et al. 2017), has been

extensively planted on the Plateau since initiation of the “Grain for Green” programme at the end of the twentieth century (Feng et al. 2005). However, long-term clean cultivation management (i.e. removal of all the inter-row plants) has led to reductions in soil quality and productivity (Gao et al. 2014). Moreover, there are frequent seasonal droughts and uneven distributions of rainfall associated with the Asian monsoon. To increase soil and water conservation capacities and improve soil fertility, farmers have been advised to plant herbaceous species between rows of jujube trees. However, little is known of the seasonal effects of intercropping and extreme drought on jujube trees’ uptake of water from both ROL and BOL.

Thus, the objectives of this study were to (1) investigate effects of intercropping on soil water profiles in different seasons, (2) determine seasonal effects of intercropping on trees’ water uptake in ROL and BOL, respectively, (3) estimate relationships between soil water profiles and plants’ water uptake. We hypothesized that compared with monocultures (1) agroforestry systems will have higher SWC in wet periods, but lower values in dry periods. (2) To cope with the low SWC and competition from intercrops in dry periods, intercropped jujube trees will absorb larger proportions of water from deeper layers of the ROL. Therefore, (3) jujube trees in agroforestry systems will show a closer correlation between the soil water profile and the trees’ water uptake pattern. Finally, (4) as an adaptive response to extreme drought, intercropped jujube trees will absorb soil water from BOL. To address these hypotheses, natural and stable-isotope-labelling techniques were used to investigate the plants’ water uptake from ROL and BOL in two early-stage (from 2 to 5 years) jujube-based agroforestry systems and a monoculture jujube plantation.

Materials and methods

Site description

The field experiment was performed at a modern agricultural base station in the middle of the Loess Plateau at 1093–1122 m a. s. l. (37°14′N, 110°21′E). At this site, mean annual precipitation is 503 mm (1956–2016), and more than 70% of the rainfall occurs from July to September. Because the loess soils (Inceptisols, according to USDA classification) are silt loams and have depths of 50–200 m, soil moisture is mainly supplied

by rainfall. The field capacity is approximately 20% (gravimetric water content), and the wilting point is approximately 5% (gravimetric water content). More details on the sites are given in Huo et al. (2018) and Gao et al. (2014).

Three jujube orchards (planted in 2008) with similar areas (ca. 2 ha), gradients (12–15°), aspects (317–355°) and positions within the slope (upper) were chosen as the experimental sites. Distances between the orchards were less than 300 m to minimize spatial variation in soil properties. The trees in the orchards were uniformly spaced, with 2.5 m between trees within rows and 6 m between rows. Two of these orchards had been intercropped with herbaceous crops between tree rows for several years. *Hemerocallis fulva* L. (*H. fulva*), an indigenous perennial species that produces edible flowers, had been grown in one since 2010. This species regenerates from roots in mid-April and senesces in late September. In the other intercropped orchard, the annual *Brassica napus* L. (*B. napus*), had been cultivated since 2013. This crop is seeded in late May, harvested in late October, and is used for oil production and to feed livestock after grain harvesting. Because of their high economic values, *B. napus* and *H. fulva* have been important cash crops in the hilly loess region. In both cases, during the study period the crops were seeded in six rows between adjacent jujube tree rows, with 0.6 m spacing within rows, and 1.5 m between the trees’ trunks and the first crop row. In the third orchard, jujube trees were kept in monoculture and all the naturally occurring grasses and shrubs were quickly removed. Thus, three treatments were applied: Jujube-*B. napus* intercropping (JB-2013), Jujube-*H. fulva* intercropping (JH-2010) and jujube cultivation in monoculture, which served as the control (JC). To ensure comparability between the intercropping and monoculture systems, most of the soil management practices affecting the water cycle were kept consistent across treatments, except that inter rows in JB-2013 were ploughed (to 20 cm deep) for seeding *B. napus* in May of each year. Meteorological data, including precipitation, air temperature and net radiation were obtained by an automatic station (AR5) installed near the JH-2010 system.

Previous studies have found that fine roots of intercrops are mainly present in the 0–20 cm soil layer, and absent below 1.2 m (Li 1999; Liu et al. 2011a), and most of the fine roots of jujube trees are also distributed above 1.2 m (Li et al. 2017). Therefore, two layers of soil were considered: the layer extending from the surface to

1.2 m, where both crops and trees may absorb water (the main Root Overlap Layer, ROL), and the layer deeper than 1.2 m (Below the main root Overlap Layer, BOL).

Experimental plot layout

Before the first sampling in 2015, three $7.5 \times 12 \text{ m}^2$ plots, each including six jujube trees (three in each of two rows), were established in orchards subjected to each treatment (JB-2013, JH-2010 and JC). To avoid damaging one tree by plant sampling, three trees with similar aboveground morphological traits (height, diameter at breast height and crown width) were marked in each plot for natural isotope analysis. In order to investigate the roots' vertical distribution of trees and crops, and determine target depths for the labelling experiment, in each 6-tree plot, one marked tree and several crops of each species (in the JH-2010 and JB-2013 orchards) were selected in September 2015. For the labelling experiment in 2016, nine trees outside of the plots ($7.5 \times 12 \text{ m}^2$) in both the JC and JH-2010 orchards were selected, with similar aboveground morphological traits and cultivation backgrounds to the selected trees in the plots. To prevent tracer injections (described below) affecting more than one selected tree, these trees were all at least 10 m apart.

Root sampling

Fine roots of jujube trees and crops were sampled by a hand auger ($\Phi = 6 \text{ cm}$) as follows. Soil samples were collected 0.5 m from the trunk of each selected jujube tree in three directions. Soil cores were taken every 0.2 m down to a depth of 3 m. Thus, 405 samples were collected in total (3 treatments \times 3 replications \times 3 directions \times 15 depths) for jujube root distribution analysis. As for intercrops, the root investigation stopped at 1.2 m and soil samples were collected from every 10 cm layer in the 0–40 cm range and every 20 cm layer in the 40–120 cm range, at 0.05, 0.10 and 0.15 m distances from marked crop plant stems. Thus, 144 soil samples were collected in total (2 species \times 3 replications \times 3 distances \times 8 depths) for crop root distribution analysis. Then, roots were obtained by washing with water and removing impurities. After the fine roots were scanned, DELTA - TSCAN image analysis software (Delta-t scan, Delta-T Devices Company, UK) was used to calculate fine root length density (FRLD, km m^{-3}) as described by Li et al. (2017).

Plant and soil sampling in the natural stable isotopes experiment

According to the long-term temporal distribution of rainfall on the Loess Plateau (Lin and Wang 2007), several stages in the growth of jujube trees can be identified: May (dry season; leaf emergence), June to July (transition from dry to wet season; blossom and young fruit), August (rainy season; fruit swelling), and September (rainy season; fruit maturation). Thus, to assess seasonal effects of intercropping on trees' use of water in ROL (Fig. 1a), plants and soil were sampled in late May (26th), early July (9th), August (8th) and September (15th) in 2015 and late May (29th), early July (6th), August (8th) and September (12th) in 2016 (Fig. 2a). Because the *B. napus* plants were too small in May, and the *H. fulva* plants were close to death in September, they were sampled only three times a year.

On each sampling date, one tree and several (2–4) crop plants were chosen in each of the three replicated plots per treatment. From each sampled tree, one lignified branch (diameter 0.1–0.3 cm) originating near the trunk was collected from both the sunny (south) and shaded (north) sides (Dai et al. 2015). As *B. napus* and *H. fulva* plants have no xylem, their roots were sampled (Barnard et al. 2006). Then, all the phloem tissues were removed to avoid contamination of the isotopically enriched water. Finally, the samples were cut into small (1–2 cm) segments and immediately placed in glass vials, sealed with parafilm and stored at -15 to $-20 \text{ }^\circ\text{C}$. The soil at six depths within the ROL (0–10, 10–20, 20–40, 40–60, 60–80 and 80–120 cm) was collected using a hand auger with an internal diameter of 5 cm, 50 and 5 cm away from the tree trunk and the crop stem, respectively. Then, each soil sample was divided into two subsamples: one portion was stored in a freezer (-15 to $-20 \text{ }^\circ\text{C}$) for isotopic analysis, while the other was used to determine the SWC by weighing before and after drying at $105 \text{ }^\circ\text{C}$ for 24 h.

Plant sampling in the isotope labelling experiment

Sets of three of the nine trees in the JC and JH-2010 orchards selected for labelling were assigned to labelling at each of three depths (2, 3 and 4 m) (Fig. 1b). To increase the probability of the presence of tree roots in the labelled zones, two holes instead of one were drilled (on May 29, 2016) to the target depth using a hand auger ($\Phi = 6 \text{ cm}$) 50 cm away

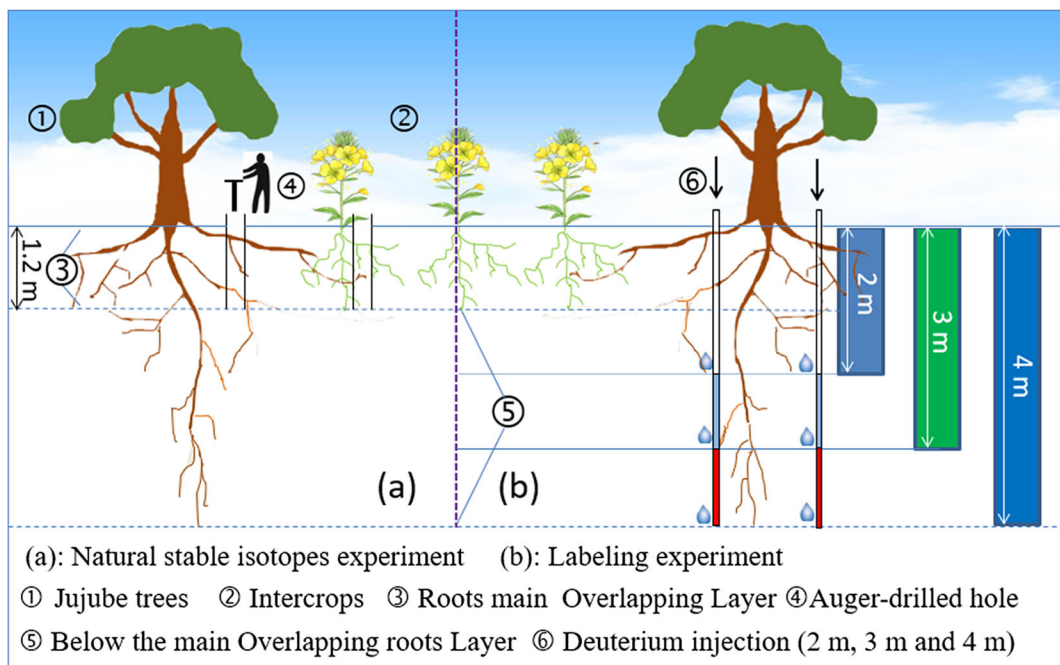


Fig. 1 Schematic visualization of the soil sampling for natural isotope analysis (a) and the tracer application via auger-drilled holes (b)

from the south and north sides of each trunk. Then, a sufficiently long polyvinyl chloride pipe ($\Phi = 3$ cm) was used to inject 300 ml tracer solution (10% $^2\text{H}_2\text{O}$, prepared by mixing 99.9% $^2\text{H}_2\text{O}$ solution and tap water, 1:9) into each hole. Preliminary analysis had shown that 300 ml of aqueous solution would wet 400 cm^3 of the soil, equivalent to a $< 1\%$ change in SWC (within the measurement error) so the impact on soil hydrological processes was negligible. Finally, the pipe was removed and the soil cores that had been removed were replaced in the corresponding holes. Since the high concentration $^2\text{H}_2\text{O}$ solution would gradually dissipate, we reopened the holes and reinjected the solution, as described above, at each of the subsequent dates (July 6, August 8 and September 12, 2016) of the natural isotope sampling experiment (Fig. 2a).

In each experiment, before the $^2\text{H}_2\text{O}$ injection three branch samples were collected from the sunny side of each tree to measure the background isotopic concentrations. As xylem flow rates may be as low as 1 m d^{-1} (Meinzer et al. 2006), triplicate samples were taken from each tree on the second and fifth days after labelling to determine whether their roots had absorbed water from the labelled zones. Plant samples were collected and preserved using the same methods as in the natural isotope tracing experiment.

Data analysis

Determination of stable isotopic composition

The cryogenic vacuum distillation method ($95\text{ }^\circ\text{C}$, 2 h) was applied to extract water from all the plant and soil samples (Orlowski et al. 2013). Then, both natural stable oxygen and hydrogen isotope ratios of the water samples collected in the natural stable isotope experiment were measured by the Liquid Water Isotope Analyzer (TIWA-45EP, Los Gatos Research (LGR), Mountain View, USA). Methanol and ethanol, which have similar spectral signals to water, may be co-extracted by the applied vacuum distillation method (Orlowski et al. 2013), so two correction equations were applied to obtain exact isotope ratios (Huo et al. 2018). As in the determination of deuterium in the labelling experiment, to reduce costs, the three samples of xylem water from each tree were pooled, then isotope ratios were measured by a Finnigan MAT Delta V advantage stable isotope ratio mass spectrometer, with an accuracy of 0.2‰ for δD and 0.025‰ for $\delta^{18}\text{O}$.

Determination of water sources

Based on the general vertical distributions of roots, SWC and isotope ratios (Huo et al. 2018), soil water

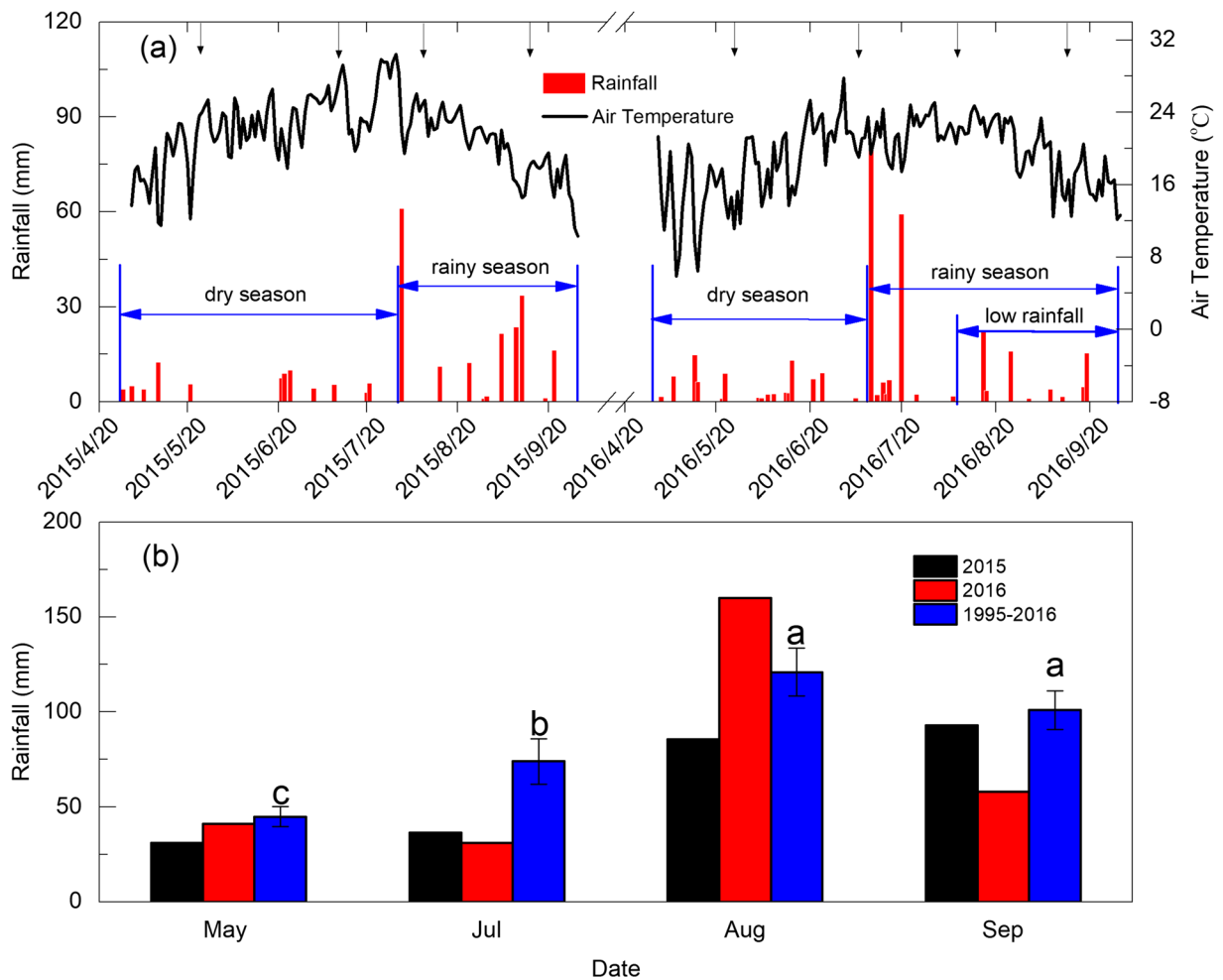


Fig. 2 Precipitation and average daily air temperature (a), total rainfall in the months before indicated sampling occasions in 2015 and 2016, and long term (1995–2016) average (mean \pm sd) (b).

Black arrows indicate the beginning of the natural and labelling-isotopic experiments. Different letters indicate significant between-period differences ($P < 0.05$)

was divided into three pooled sources: 0–20, 20–60 and 60–120 cm. A Bayesian mixing model implemented in the SIAR package in R (Parnell et al. 2008) was applied to calculate the proportional contribution of each source for each plant, using the means and standard deviations of both isotope ratios ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) in soil and plant water as model inputs. As no fractionation occurs during water uptake from the soil by roots (Ehleringer and Dawson 1992), both the concentration dependence and trophic enrichment factor (TEF) were fixed at zero. The number of iterations was set to 500,000, and the number of initial iterations to discard was fixed to 50,000 using Markov chain Monte Carlo (MCMC) methods (Wang et al. 2019).

In the labelling experiments, following Kulmatiski et al. (2010), we inferred the presence of the ^2H tracer in

a xylem sample if its concentration in xylem water was at least two standard deviations (SD) higher than the background value. Therefore, in each sampling period, we calculated the SD from measurements of all xylem water samples collected before the $^2\text{H}_2\text{O}$ injection, then plotted a line two SD above the maximum background value to judge whether the injected stable deuterated water ($^2\text{H}_2\text{O}$) was absorbed by jujube trees.

Determination of relationships between soil water profiles and water use patterns

Intercropping may change the soil water profiles, and thus the soil water amount ratios (relative to the entire profile) of each specific layer. Therefore, to test the third hypothesis, the gravimetric SWC of each layer

(obtained in the “*natural stable isotopes experiment*” by drying) was converted into volumetric SWC based on the soil bulk density, and the amount of soil water in each layer was calculated according to their thickness. Then the amount of water in each of the three mentioned sources (0–20, 20–60 and 60–120 cm) and the entire profile (0–120 cm) was summed and used to calculate the relative soil water amount ratio of each source.

Statistics

One-way analysis of variance (ANOVA) was used to examine the differences in SWC and proportional contributions of sources between treatments. Significant between-treatment differences in SWC and proportional contributions were identified using *t*-tests ($P < 0.05$). Linear regression was used to analyse the correlations between the proportional contributions of each water source (0–20, 20–60 and 60–120 cm soil layers) to plants’ water uptake and their water amount ratios. All statistical analyses were performed using SPSS 22.0 and Origin 2016 software packages, and all the graphics, except Fig. 1, were generated using Origin 2016.

Results

Precipitation, soil water conditions and root distribution

Precipitation from May to September amounted to 255 mm and 319 mm for 2015 and 2016, respectively (35 and 21% lower than the long-term mean of 407 mm). The long-term average precipitation in May is just 45 mm (Fig. 2b). And the precipitation in the month before sampling in July amounted to just 35 and 31 mm, in 2015 and 2016, respectively (53 and 58% lower than the long-term means in the corresponding periods, Fig. 2b). However, the monthly rainfall before sampling in August and September in both years was clearly higher than the monthly rainfall before sampling in May and July, except in September 2016, when the rainfall one month before sampling was 58 mm, 42% lower than the long-term mean in the corresponding period (Fig. 2b). Therefore, conditions were extremely dry on the sampling occasions in May and July for both years, and September 2016, but relatively wet in August for both years and September 2015.

Under all treatments, SWC in the 0–20 cm and 20–60 cm layers was obviously affected by seasonal drought (Fig. 3). In both years, as the drought lasted from May to July, SWC in the 0–20 cm and 20–60 cm layers declined. Then, the 0–60 cm layers in the agroforestry systems were recharged during the rainy season (August in both years and September in 2015, Fig. 3a, b, c, d). However, except for August 2016, SWC in the 60–120 cm layers showed downward trends over time (Fig. 3e, f). In addition, intercropping significantly changed the SWC in the jujube orchards, especially in the 20–60 and 60–120 cm layers. The SWC in the 20–60 cm layer was significantly higher in the JH-2010 and JB-2013 plots than in JC plots in August 2015 and 2016 (Fig. 3c, d). However, in most periods, intercropping reduced the SWC in the 60–120 cm layers (Fig. 3e, f). Both *B. napus* and *H. fulva* had much higher FRLD than the jujube trees, but the trees’ roots extended deeper into the soil (Fig. 4). Specifically, the FRLD of the jujube trees gradually decreased with increases in soil depth, and most of the fine roots were concentrated in ROL (Fig. 4a). As for the fine roots of *B. napus* and *H. fulva*, most of them were present in the 0–20 cm layer, and their densities were dramatically lower at 20–30 cm depth (Fig. 4b).

Determination of plant-water sources in ROL

The soil water use patterns of jujube trees exhibited clear seasonal changes and were sometimes significantly affected by intercrops. In the extremely dry May in both years, they took up a small proportion of water from the 60–120 cm layer in JC plots (Fig. 5a, g), but intercropping significantly increased this proportion in 2015 (Fig. 5a). In contrast, during the extremely dry July of both years, the trees in JC plots absorbed ca. 50% of their water from the 60–120 cm layer, and the trees in JH-2010 and JB-2013 plots took up non-significantly higher ($P > 0.05$) proportions from it (Fig. 5b, d, h, j). During the rainy season (August and September) in 2015, the soil water in 20–60 cm layers became the main source for jujube trees under all treatments (Fig. 5c, e, f), except those in agroforestry systems in August, when they absorbed higher proportions of water from the 0–20 cm layer and lower proportions from the 20–60 cm layer than trees in JC plots (Fig. 5c, e). However, in 2016, intercropping increased the proportional contribution of water in the 20–60 cm layer to trees’ uptake in the wet August (Fig. 5i, k), and increased the

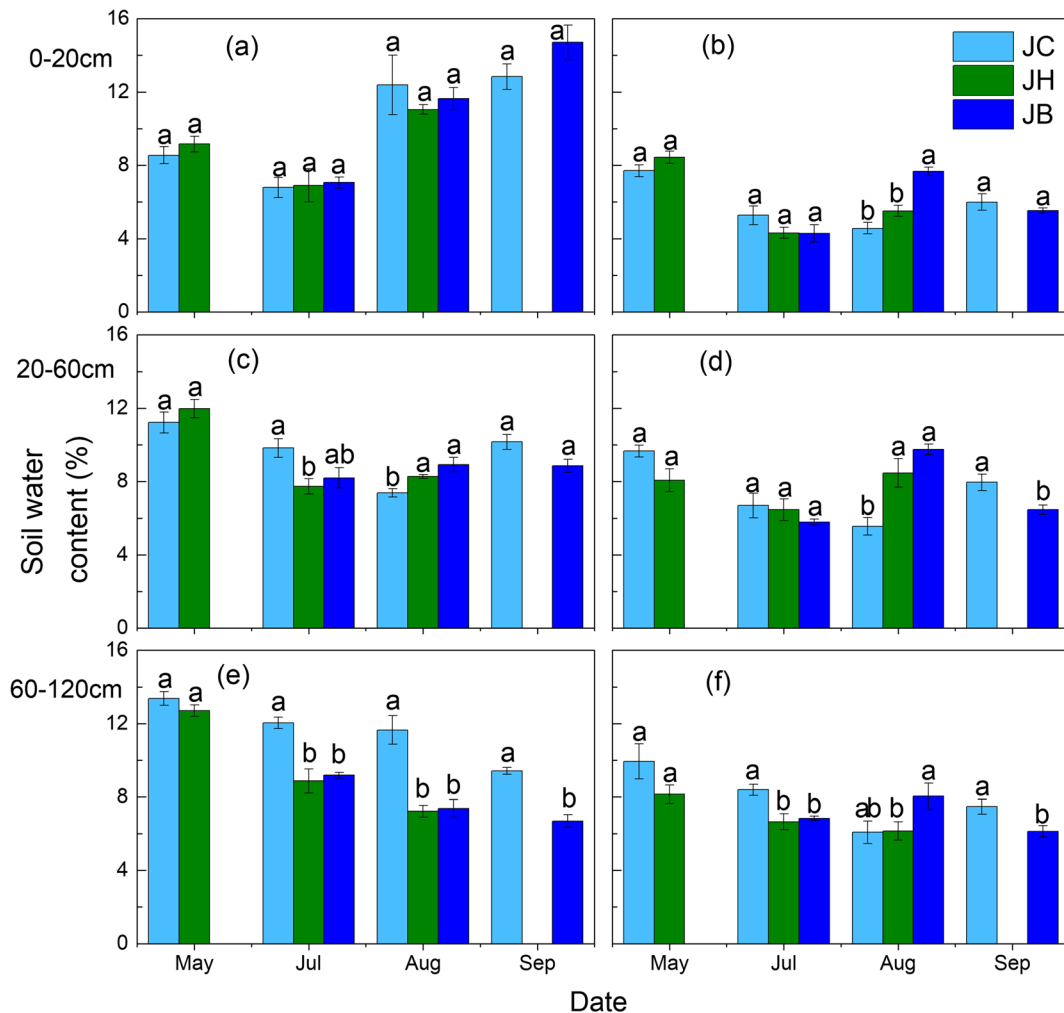


Fig. 3 Seasonal variations in gravimetric soil water content (mean \pm se) in indicated layers under each of the treatments in 2015 (left) and 2016 (right). Different letters indicate significant between-treatment differences ($P < 0.05$)

proportional contribution of the water in the 60–120 cm layer during the extremely dry September (Fig. 5I). We also calculated proportional contributions of the soil water in 0–20, 20–60 and 60–120 cm layers to the intercrops. There were significant differences between the water use patterns of crops and associated trees in most periods, except in August in both years (Fig. 5c, e, k).

Relationships between soil water profiles and water use patterns

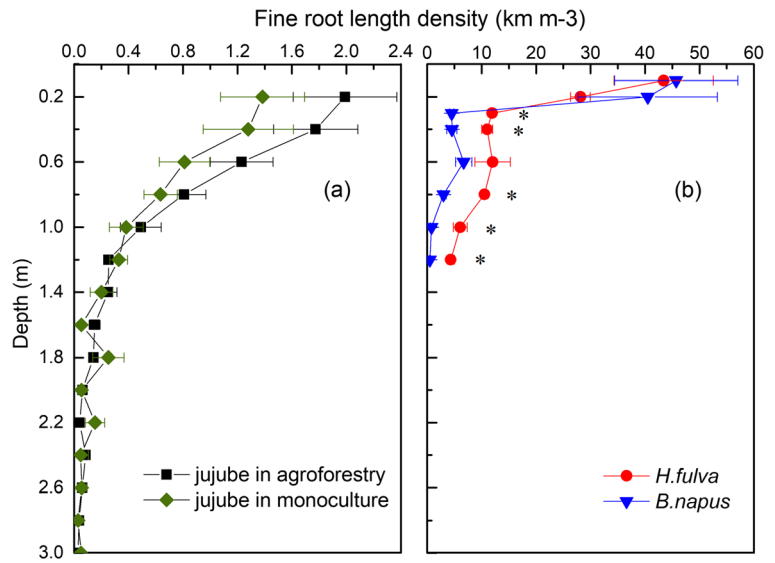
Intercropping changed the relationships between the sources' water amount ratios, which relative to the entire profile, and their proportional contributions to trees' water uptake (Fig. 6). There was a highly significant

($P < 0.001$) positive relationship between these two variables for jujube trees in both agroforestry systems and in both years, but not in the monoculture (JC). In contrast, the soil layers' proportional contributions to both intercrops were significantly negatively correlated with their soil water amount ratios, except for *H. fulva* in 2015.

Water uptake patterns in BOL

Generally, the artificial tracer was only found in xylem water on the second day after the labelling. Uptake of $^2\text{H}_2\text{O}$ by trees in JH-2010 and JC plots was tracked to depths of 2 and 3 m respectively, but there was no clear signal of uptake from 4 m except for trees in JH-2010 plots in July (Fig. 7f). No artificial tracer was detected in

Fig. 4 Vertical distributions of the fine root length density (mean \pm SD) of the plant species in the agroforestry systems and jujube monoculture. Asterisks (*) indicate significant differences between *B. napus* and *H. fulva* ($P < 0.05$)



xylem water samples under either the JH-2010 or JC treatments in May (Fig. 7a, e). However, high

concentrations of artificial ^2H were detected in xylem samples from JH-2010 plots after injections at depths of

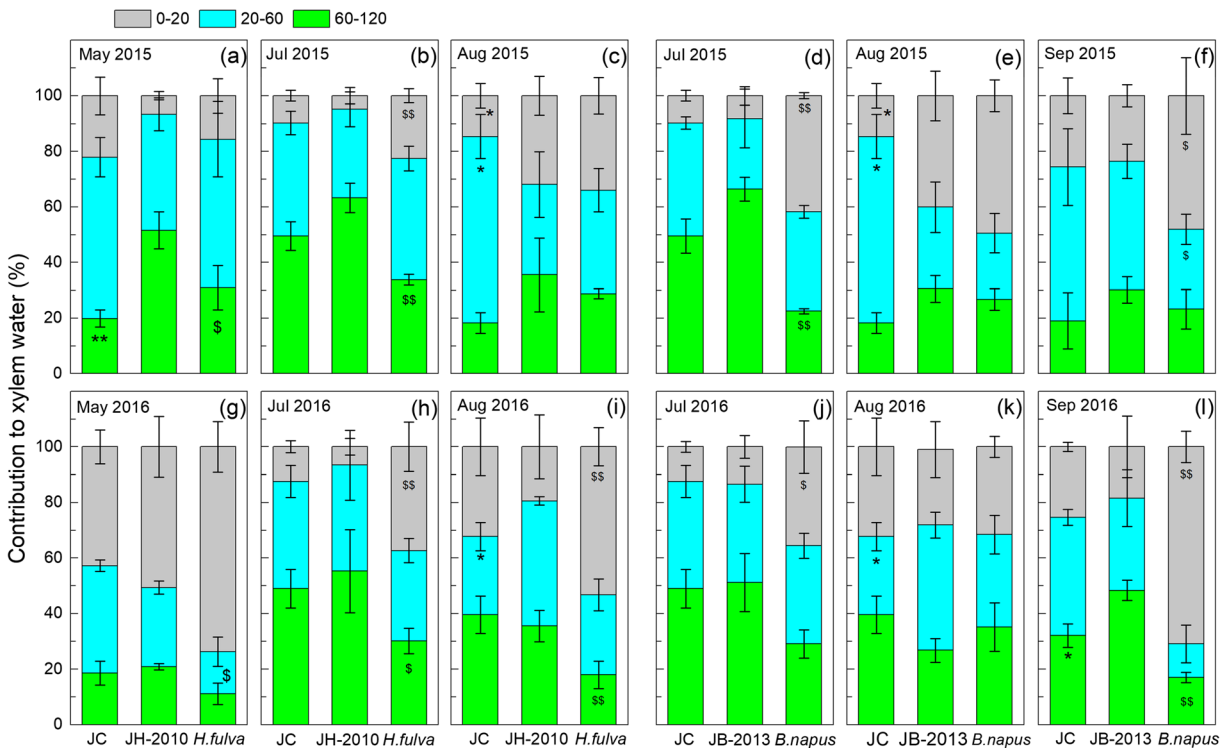


Fig. 5 Seasonal variations in proportion (mean \pm se) of water uptake by the jujube trees and intercrops from indicated water sources (0–20, 20–60 and 60–120 cm) according to the model implemented in SIAR. One and the same JC were synchronously used to compare with the two intercropping systems. Asterisks indicate significant differences between jujube trees in

monoculture (JC) and the presence of intercrops (JB-2013, with *Brassica napus*, and JH-2010, with *Hemerocallis fulva*). Dollar symbols indicate significant differences between jujube trees and the associated intercrops in the agroforestry systems (* or \$, $P < 0.05$; ** or \$\$, $P < 0.01$)

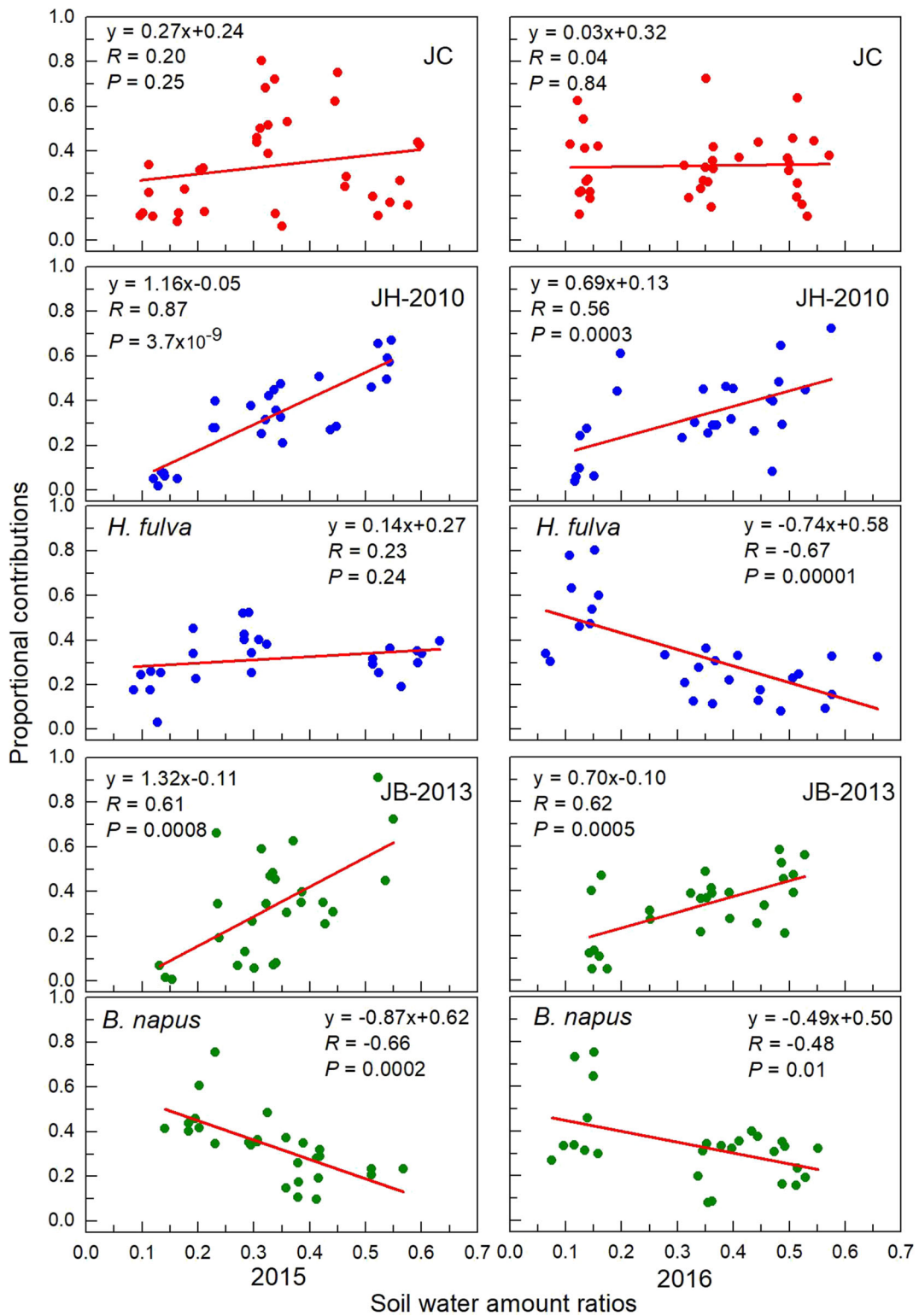


Fig. 6 Relationships between indicated soil layers’ water amount ratios (relative to the total water amount of the entire soil profile) and their proportional contributions to water uptake of jujube trees in

monoculture (JC) and the two agroforestry systems (JH-2010 and JB-2013) and the two species of intercrops (*H. fulva*, *B. napus*) in 2015 (left) and 2016 (right), based on the data from all sampled plants

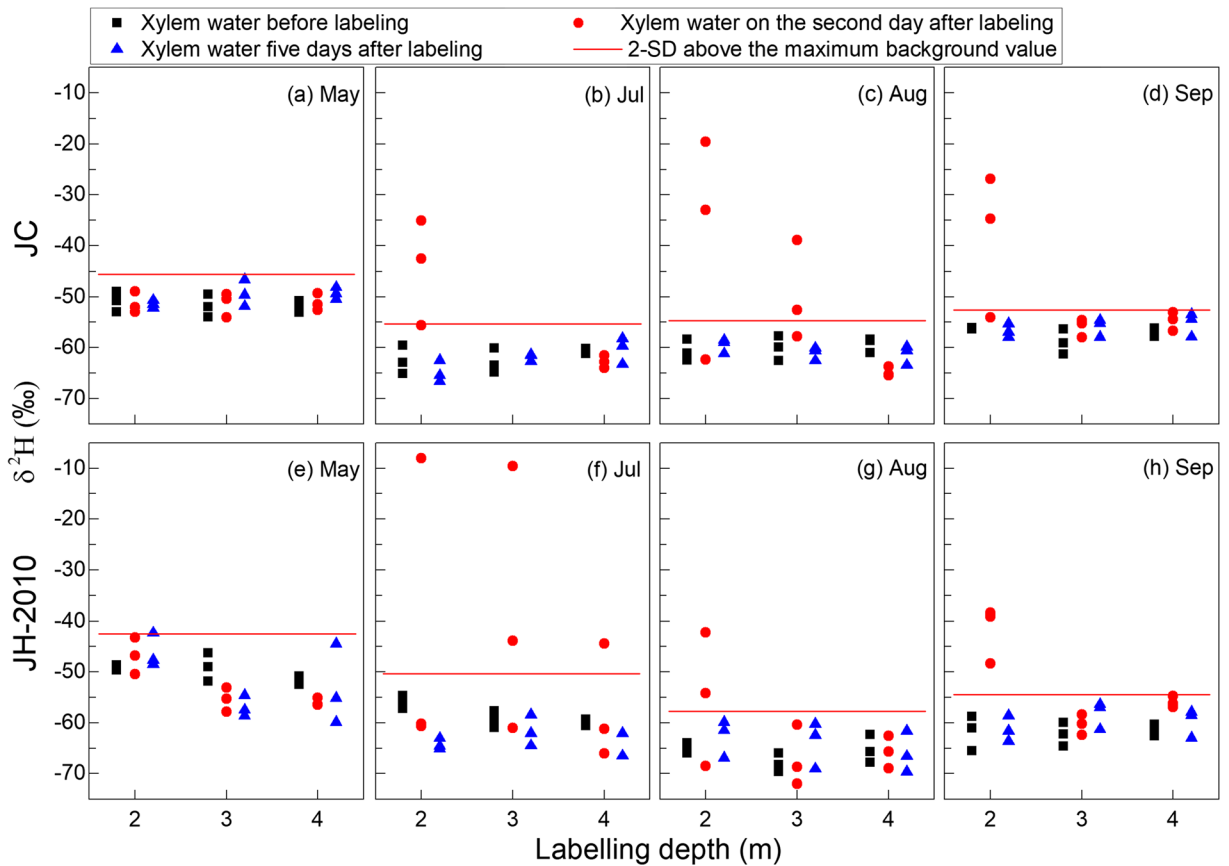


Fig. 7 $\delta^2\text{H}$ of all xylem samples obtained from trees in jube monoculture (JC, upper panels) and with intercropped *Hemero-callis fulva* (JH-2010, lower panels) in the labelling experiment.

The solid red lines represent two-SD (calculated from the $\delta^2\text{H}$ in xylem water sampled before the $^2\text{H}_2\text{O}$ injection) above the maximum background value

2, 3 and 4 m in July, when artificial tracer was only found in samples from JC plots after labelling at 2 m (Fig. 7b, f). In contrast, the maximum detected depth of $^2\text{H}_2\text{O}$ uptake was shallower in JH-2010 plots (2 m) than in JC plots (3 m) in the wet August (Fig. 7c, g). In the extremely dry period of September, labelling at 2 m depth led to high concentrations of ^2H in xylem samples from trees in both treatments, but not labelling at 3 or 4 m (Fig. 7d, h).

intercropping effectively promotes infiltration of heavy rainfall. However, in most of the extremely dry periods, intercropping reduced the SWC in the deeper layers. Moreover, except for August 2016, this part of SWC declined with time (Fig. 3e, f). On the one hand, this may have been mainly due to competition from the intercrops inducing increases in the jujube trees’ absorption of deep soil water. On the other hand, this indicates that deep water will be continuously absorbed by jujube trees in dry years and will not be effectively supplemented by rainfall.

Discussion

Seasonal effects of intercropping on soil water contents

In general, our results supported the first hypothesis. In the wet Augusts of both years, intercropping promoted the replenishment of soil water in the upper soil layers in both agroforestry systems (Fig. 3b, c, d), indicating that

Seasonal effects of intercropping on jujube trees’ patterns of water use in ROL

The second hypothesis was partially supported by the results. In dry periods, intercropping indeed increased the utilization of deeper (60–120 cm) soil water by jujube trees (Fig. 5a, b, d, h, l). This was similar to the

findings of several other studies, which reported that trees could shift water sources to deep soil layers to cope with drought (Liu et al. 2011b; Yang et al. 2015; Wu et al. 2016, 2017). In this study, on the one hand, jujube trees in the JC plots showed high variability in their preferred depths for water uptake under different soil water conditions (Fig. 5c, g, h), which indicates that jujube trees have dimorphic root systems. On the other hand, most fine roots of intercrops were distributed in the 0–20 cm layer and several-fold higher than that of jujube trees (Fig. 4). This suggests that jujube trees need to shift to use of deeper soil water, thereby avoiding competition with intercrops when SWC in shallow layers is low (Fig. 2a, b). This was supported by the results that water use patterns of intercrops were significantly different with jujube trees in dry periods (Fig. 5a, b, d, g, h, j and l). However, in July of two years, although intercropping caused trees to absorb higher proportions of water from deeper (60–120 cm) soil layers (Fig. 5b, d, h, j), the enhancement was not as significant as in May 2015 and September 2016 (Fig. 5a, l). This may have been partly due to extremely low SWC in the entire profile, especially in July 2016 when the SWC approached the wilting point (5%) (Fig. 3). Another possible contributory factor is that jujube trees have higher water requirements in July than in September (Xin et al. 2012), suggesting that jujube trees may lose the ability to adapt their water absorption from ROL in response to effects of intercrops and extreme drought when their water requirements are high and the SWC falls below a certain threshold.

In wet periods, the effects were mainly localized in the upper layers (0–20 or 20–60 cm). Similar phenomena have been observed in other agroforestry systems and attributed to the water availability in shallow soil layers (Wu et al. 2017). Hence, it is easy to understand that intercropping induced the trees in agroforestry systems to absorb proportionally more water from the 20–60 cm layer in August 2016 (Fig. 5i, k), because of higher SWC at the 20–60 cm depth in agroforestry systems than in monoculture (Fig. 3d), and lower SWC in the 0–20 cm layers than in the 20–60 cm layers (Fig. 3b, d). However, in August 2015, the presence of intercrops increased the proportion of water trees absorbed from the 0–20 cm layer (Fig. 5c, e), even though there was no significant difference in SWC between JC and JH-2010 or JB-2013 plots (Fig. 3a). In this case, possibly because the interception of radiation by intercrops reduced the soil temperature, enabling the

trees to maintain strong root activity (Williams and Ehleringer 2000).

Relationships between soil water profiles and water uptake patterns of the plants

There were significantly positive correlations between sources' water amount ratios and their proportional contributions to the trees' water uptake in both JH-2010 and JB-2013 plots in both years (Fig. 6), which strongly supports our third hypothesis, and was probably due to the competition from intercrops. Clearly, the strong concentration of the crops' roots in the 0–20 cm layer largely restricted their water absorption to shallow soil layers, even in extremely dry periods (Fig. 4b and Fig. 5). This also explains why the relationships between sources' proportional water amount ratios and proportional contributions to water uptake were not positive, or even negative, for intercrops. In addition, the several-fold higher FRLD of the intercrops than jujube trees indicates that they are more competitive, especially in extremely dry seasons, when the interspecific competition is most intense (Sanchez 1995). All of these findings suggest that jujube trees in agroforestry systems have to take up water from soil layers with relatively high proportional water amount ratio. However, there was no such correlation for jujube trees under the JC treatment. Similarly, Nnyamah and Black (1997) found that plants' water uptake patterns did not always match the root distribution and soil water profile. A possible explanation is that the plants' water requirements can be more easily met in JC plots, than in agroforestry systems where there are both trees and crops. In this case, water use patterns of trees may be affected by both root and soil water distribution, and it is difficult to determine which is the dominant factor. Thus, further investigation is needed to disentangle effects of root and soil water distributions on trees' water uptake.

Effects of intercropping on jujube trees' patterns of water use in BOL

Consistent with our fourth hypothesis, intercropping promoted jujube trees to absorb water from BOL, even to 3 or 4 m (Fig. 7b, f). It should be noted that Beyer et al. (2016) detected no artificially injected ^2H in plants when tracer was added at depths greater than 2 m in semiarid environments, and postulated that it may have been at least partly because the sampled plants' lateral

roots were at most 2 m deep. However, the presence of jujube roots at 3 m depth was confirmed by coring techniques in our experiment (Fig. 4a, b), and high concentrations of $^2\text{H}_2\text{O}$ were detected in two of the three replicates. Thus, we conclude that intercropping can promote absorption of deeper soil water by trees in some extremely dry periods. However, since no data on roots at 4 m are available, it is difficult to tell whether the presence of signals from labelling at 4 m in the JH-2010 system, but not the JC system, was due to effects of intercropping in the former or absence of sufficiently deep roots in the latter. But at least it can be proven that jujube trees can absorb water from 4 m depth in adaptive responses to extreme drought. In the dry May and September, there was no effect of intercropping on the trees' water absorption (Fig. 7a, e, d, h). This may have been because jujube trees have lower water requirements in May than in other growth stages (Xin et al. 2012), and SWC in the 20–120 cm layer was still high due to replenishment in winter by snowfall (Fig. 3c, e). And in September, *H. fulva* plants were close to death and were not competing for soil water with jujube trees. Conversely, relative to jujube trees in monoculture, intercropping encouraged jujube trees to absorb shallower soil water (in the BOL) in the wet August (Fig. 7c, g). This was mainly because enhancement of infiltration by intercrops increased SWC in the ROL (Fig. 3b, d, f), thus reducing the jujube trees' dependence on deeper water sources.

Implications for future vegetation management

Our study showed that in extremely dry conditions intercropping induced jujube trees to absorb deeper soil water, an adaptive response that enabled them to meet their growth requirements and cope with the intercrops' competitive effects. Such responses are clearly beneficial for the trees' survival. However, in the Loess Plateau, groundwater is extremely deep (generally >50 m; Gao et al. 2014; Wang et al. 2017), and difficult to replenish when the deep soil water has been depleted (Li et al. 2008). This suggests that the prolonged use of deep soil water may lead to desiccation. Fortunately, in the rainy season intercropping prompted jujube trees to use more water in the 0–20 or 20–60 cm layers (Fig. 5c, e, i, k) and reduced their depth of water absorption in BOL (Fig. 7c, g). These findings indicate that we need to choose intercrops that

grow in the rainy season and increase the soil's infiltration capacity. However, relying solely on species selection seems to be insufficient, because we found that the soil water at 60–120 cm depth was not well replenished by the large rainfall in the rainy season in 2015 (Fig. 3e). Hence, constructing water-fertilizer pits, which can deliver rainwater directly to deep layers and effectively increase the SWC (Song et al. 2017), may be a good choice. Moreover, increases in temperature on the Loess Plateau are predicted (Huang et al. 2017), which would increase evaporation. In such cases, the timely cutting of intercropped crops and using them as mulch under trees may be highly beneficial as it would not only reduce interspecific competition and evaporation but also increase soil fertility.

Conclusions

This study investigated the seasonal effects of intercropping on jujube trees' water use strategies at the early stage of land-use change from plantation to agroforestry, using a method combining natural and stable-isotope-labelling techniques. Relative to monoculture, intercropping affected the water use strategies of jujube trees in both ROL and BOL. More specifically, in ROL, intercropping induced jujube trees to obtain higher proportions of water from shallow (0–20 cm or 20–60 cm) layers during wet periods, but more from deeper layers (60–120 cm) during dry periods. In addition, intercropping induced jujube trees to absorb higher proportions of water from layers with higher proportions of total water contents. In extremely dry periods with high water demands, intercropping even promoted jujube trees' absorption of soil water from 3 m underground. This indicates that soil water in BOL is an important buffer that enables trees to cope with extreme drought and the water competition from intercrops in agroforestry systems. The results provide insights into effects of intercropping on the water uptake dynamics of trees in whole vertical root profiles. However, different lengths of intercropping history limited the comparison of the effects caused by different intercrop species. Besides, the effects of long-term intercropping on

tree water use need further investigations to facilitate formulations of appropriate sustainable amelioration strategies for degraded systems.

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