



Seasonal variations in depth of water uptake for a subtropical coniferous plantation subjected to drought in an East Asian monsoon region



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ABSTRACT

Seasonal drought will become more intense and frequent in some regions due to global climate change, which may lead to significant changes in the competitive interactions and uptake depths of plant water sources. Based on $\delta^{18}\text{O}$ and δD in xylem and soil water, we investigated the impacts of seasonal drought on plant water sources from July 2011 to October 2013 for a subtropical coniferous plantation in south-eastern China. Our results indicated that the studied tree species of *Pinus massoniana*, *Pinus elliottii* and *Cunninghamia lanceolata* appeared to have inter-specific competition for water resources from similar depths. There was a switch of the major water source from shallow soil during the non-drought periods (July to October) to deep soil during the drought periods (November–June). Similar seasonal trend of water uptake was estimated by $\delta^{18}\text{O}$ and δD . However, relative to the results of δD , water sources predicted by $\delta^{18}\text{O}$ showed that trees seemed to derive more deep soil water during the drought periods and more shallow soil water during the non-drought periods. The differences of plant water source partitioning based on $\delta^{18}\text{O}$ and δD were significant during both the drought and non-drought periods, which probably resulted from the artifacts of the cryogenic vacuum distillation and spectral contamination correction. These findings will have important implications for further studies when just one of the dual stable isotopes of $\delta^{18}\text{O}$ and δD is applied.

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1. Introduction

Forests play an important role in terrestrial ecosystem hydrological cycles, and the distribution and abundance of which are mainly constrained by water resources (Dodd et al., 1998; Drake and Franks, 2003; Liu et al., 2010a,b). As anthropogenic influence has led to significant changes in climate, the occurrence of drought will become more intense and frequent in the future (IPCC, 2013). During the drought periods, coexisting plants usually adopt contrasting water use strategies, such as temporal and spatial partitioning of resource utilization, to maximize species diversity (Dodd et al., 1998; Liu et al., 2010a,b; Moreno-Gutiérrez et al., 2012). It also has been suggested that species with dimorphic root morphology could tap distinct zones of soil water, and change source water utilization seasonally

(Ehleringer and Dawson, 1992; Meinzer et al., 1999; Nie et al., 2011). The hydrogen and oxygen stable isotopes ($\delta^{18}\text{O}$ and δD) provide us a powerful tool for determining plant water sources in a number of environmental conditions (Dawson and Ehleringer, 1991; Eggemeyer et al., 2009; Brooks et al., 2010; West et al., 2012).

Plant species with contrasting water use strategies are shown to coexist in different ecosystems (Eggemeyer et al., 2009; Liu et al., 2010a,b; Moreno-Gutiérrez et al., 2012). For example, mixed woody–herbaceous systems are able to exist, mainly because trees and shrubs tend to utilize deeper soil water than grasses (Le Roux et al., 1995; Asbjornsen et al., 2008; Eggemeyer et al., 2009). Studies of comparing depths of water uptake by co-occurring woody species show that some species only tap water from deep or shallow soil, while others use both layers (Dodd et al., 1998; West et al., 2007; Eggemeyer et al., 2009). The different water use strategies of coexisting species can be considered as a hydrological niche partitioning, which plays a positive role in minimizing competition and improving adaptability in water-limited ecosystems (Moreno-Gutiérrez et al., 2012).

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Plants capable of absorbing deeper soil water should be more competitive than those of solely depending on surface soil water, due to the severe water deficit of surface soil during drought periods (Ehleringer and Dawson, 1992). As rainfall is plentiful in tropical and subtropical forests, plants are normally shallow rooted and extract water from surface soil layers (Schenk and Jackson, 2005). In water limited regions, where surface soil water tends to be unavailable, plants are generally deep rooted and primarily tap deep soil water (Nie et al., 2011). But in fact, many plant species in arid and semiarid ecosystems, as well as in seasonal dry areas, possess a dimorphic root system: a zone of lateral roots predominantly acquires water from surface soil layers in wet season, and a second zone of penetrating taproots derives water from deep soil layers in dry season (Ehleringer and Dawson, 1992; Dawson and Pate, 1996; Liu et al., 2010a,b).

A number of previous studies have been conducted in arid and semiarid regions; however, plant water sources in tropical and subtropical areas are little known (Liu et al., 2010a,b; Goldsmith et al., 2011; Nie et al., 2011). This is mainly because the premise of identifying plant water sources by stable isotopes is that there must be clear vertical gradients of $\delta^{18}\text{O}$ and δD in soil stratum (Ehleringer and Dawson, 1992). But the $\delta^{18}\text{O}$ and δD in precipitation often exhibit little seasonal variations in tropical and subtropical regions compared to that in temperate areas, which leads to the similar signatures of $\delta^{18}\text{O}$ and δD in soil stratum (Querejeta et al., 2007). Moreover, most of previous studies utilize just one of the dual stable isotopes of $\delta^{18}\text{O}$ (McCole and Stern, 2007; Querejeta et al., 2007; Liu et al., 2010a,b; Nie et al., 2011) or δD (Jackson et al., 1995; Filella and Peñuelas, 2003; West et al., 2012). Although $\delta^{18}\text{O}$ and δD are both used in some studies (Cramer et al., 1999; Li et al., 2007; West et al., 2007; Eggemeyer et al., 2009; Wang et al., 2010; Rossatto et al., 2012; Orłowski et al., 2013; Meißner et al., 2014), whether there are differences between $\delta^{18}\text{O}$ and δD predictions of water source are still unclear.

According to the 7th National Forest Resource Inventory Report, plantations occupy about 31.6% of the total forest area in China, ranking 1st in the world (CAF, 2010). Furthermore, 54.3% of the plantations are distributed in the subtropical region (Sun et al., 2006). This region is characterized by the subtropical Eastern-Asian monsoon with abundant water and energy, however, seasonal drought always occurs resulting from the inconsistent distributions of temperature and precipitation in summer (Wen et al., 2006, 2010; Tang et al., 2014a,b). Previous studies mainly focused on the effects of seasonal drought on carbon and water exchange processes for the coniferous plantation at Qianyanzhou site in ChinaFLUX (Wen et al., 2006, 2010; Saigusa et al., 2010; Tang et al., 2014a,b). The objectives of this study are to (i) investigate the competitive interactions of water sources for tree species of Masson pine (*Pinus massoniana* Lamb.), Slash pine (*Pinus elliottii* Englem.) and Chinese fir (*Cunninghamia lanceolata* Hook.), (ii) analyze the impacts of seasonal drought on water uptake depth for trees, (iii) identify whether there are differences between $\delta^{18}\text{O}$ and δD predictions of water source.

2. Materials and methods

2.1. Study site

Qianyanzhou site, a member of ChinaFLUX, is located in Qianyanzhou ecological station of the Chinese Ecosystem Research Network (CERN) (26°44'52"N, 115°03'47"E, and elevation 102 m). This area is strongly influenced by the subtropical Eastern-Asian monsoon climate. Annual precipitation and mean air temperature are 1377.4 mm and 17.1 °C (1985–2013), according to meteorological records of CERN. The soil is mainly red earth, which is weathered

from red sandstone and mud stone (Wang et al., 2011). Soil texture is divided into the following particle grades: 2.0–0.05 mm (17%), 0.05–0.002 mm (68%) and <0.002 mm (15%). Bulk density of the surface soil (0–40 cm) is 1.57 g cm⁻³. The coniferous plantation was planted around 1985, and the prevailing tree species were *P. massoniana* Lamb., *P. elliottii* Englem. and *Cunninghamia lanceolata* Hook., with sparsely scattered woody species of *Schima superba* Gardn. et Champ. According to the survey conducted in 2008, mean heights of *P. massoniana*, *P. elliottii* and *C. lanceolata* were 11.2, 14.3 and 11.8 m, mean diameters at breast height were 13.6, 18.2 and 13.8 cm, and the stem densities were 700, 545 and 93 stems ha⁻¹, respectively. Further details about the site are provided in Wen et al. (2006, 2010) and Tang et al. (2014a,b).

2.2. Meteorological measurements

Supporting measurement consisted of a suite of micrometeorological sensors installed above the canopy and in the soil. They provided half-hourly measurement of net radiation (Model CNR-1, Kipp & Zonen Inc.), air temperature (Model HMP45C, Vaisala Inc.), soil water content (Model CS615-L, Campbell Scientific Inc.), soil heat flux (Model HFT-3, Campbell Scientific Inc.), and precipitation (Model 52203, RM Young Inc.).

Budyko's aridity index (AI) is calculated by the ratio of precipitation amount to potential evapotranspiration (P_{ET}). AI < 1 indicates periods of drought stress (Budyko, 1974). P_{ET} was calculated as 1.26 × the equilibrium evapotranspiration (ET_{eq}) (Priestley and Taylor, 1972), which was calculated as

$$ET_{eq} = \frac{((R_n - G) \times s)}{(s + \gamma)} \quad (1)$$

where R_n is the net radiation (W m⁻²), G is the soil heat flux, s is the slope of a function relating saturation vapor pressure to temperature (kPa K⁻¹), γ is the psychrometric constant (kPa K⁻¹).

2.3. Water sampling and isotopic analyses

Tree stems and soils were sampled over a twenty-eight month period from July 2011 to October 2013 for determining their $\delta^{18}\text{O}$ and δD . Stems were sampled from the south side of one mature tree per species (*P. massoniana*, *P. elliottii* and *C. lanceolata*) every 2–4 days per week (at midday). These trees were randomly selected around a bamboo building (~12 m height), which was used to facilitate the sampling of tree twigs. Stems samples for *C. lanceolata* were sampled only once per week since April 2012, because the continuous sampling was more destructive to this tree species. For each sample, phloem tissue was removed to avoid contamination by isotopically enriched water (Querejeta et al., 2007). Then, the stems were immediately cut into small segments, placed in vials and sealed with parafilm.

One soil core of 0–100 cm (increased to 3 soil cores since January 2013) was sampled monthly by a hand auger around the sampling trees. The soil core was collected every 5 cm depth in the 0–20 cm, every 10 cm in the 20–60 cm and every 20 cm in 60–100 cm layers. The three tree species were planted together and mixed well, therefore, we assumed the $\delta^{18}\text{O}$ and δD in soil water around the sampling trees were consistent.

A funnel and a polyethylene bottle were connected together as a rain collector. A ping-pang ball was put in the funnel to prevent evaporation (Liu et al., 2010a,b). Precipitation samples were collected after each rain event.

All the samples for isotopic analyses were kept frozen in a refrigerator (–15 to –20 °C) prior to water extraction. Water in xylems and soil samples were extracted using a cryogenic vacuum distillation system (West et al., 2006). Generally, 0.5–1.5 h would be required depending on the water content of samples. The extracting

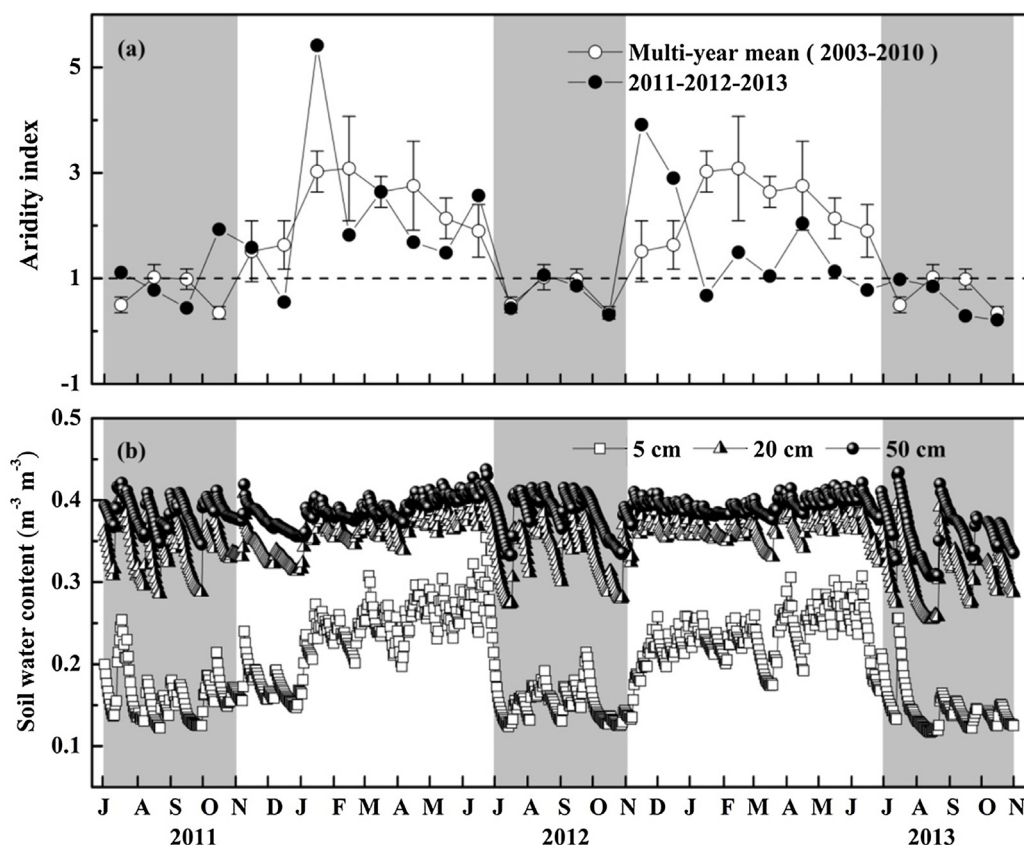


Fig. 1. Seasonal variations of (a) monthly Budyko'aridity index (AI) and (b) soil water content (SWC) between July 2011 and October 2013 at the experimental site. Error bars represent 1 SE. Multi-year mean values of monthly AI between 2003 and 2010 are also presented. Shadow areas indicate the seasonal drought periods (July–October).

percent of water from samples were >99.0%, which would be sufficient to obtain un-fractionated water samples (West et al., 2006; Meißner et al., 2014). Isotopic composition of the liquid samples was analyzed with an Isotopic Ratio Infrared Spectroscopy (IRIS) system, (Model DLT-100; Los Gatos Research, Mountain View, CA, USA). The precision of the liquid water isotope analyzer was typically better than 0.1‰ for $\delta^{18}\text{O}$, and 0.3‰ for δD (Wen et al., 2012). Due to the organic contaminants of water cryogenically extracted from plant tissues, $\delta^{18}\text{O}$ and δD of xylem water measured by the LGR system were corrected following the procedure of Schultz et al. (2011) and Xiao et al. (2012). The average corrections for $\delta^{18}\text{O}$ and δD were $1.5 \pm 0.1\%$ and $2.8 \pm 0.1\%$, respectively.

Monthly weighted means of $\delta^{18}\text{O}$ and δD for precipitation ($\delta_{p,\text{mean}}$) were calculated as

$$\delta_{p,\text{mean}} = \frac{\left(\sum_{i=1}^n \delta_{p,i} \times PPT_i\right)}{\left(\sum_{i=1}^n PPT_i\right)} \quad (2)$$

where $\delta_{p,i}$ is the $\delta^{18}\text{O}$ and δD of the i th precipitation, PPT_i is the amount of the i th precipitation.

2.4. Water source partitioning

The Bayesian-mixing model (MixSIR 1.0.4) was used to determine the uptake fractions of water sources (Moore and Semmens, 2008). The fundamental construct of this model is based on mass balance. Therefore, its performance is subject to many previous mixing models, such as the IsoSource model (Phillips et al., 2005). MixSIR incorporates a variety of sources of uncertainty (e.g., variability in isotope signatures, isotope fractionation and multiple sources). In this study, the individual xylem isotope values of different trees (*P. massoniana*, *P. elliotii* and *C. lanceolata*), the means and

SD (standard deviation) of isotope values from different soil layers (0–5, 5–10, 10–15, 15–20, 20–30, 30–40, 40–50, 50–60, 60–80 and 80–100 cm) were used as inputs to MixSIR. Since only one soil core was sampled monthly during the first eighteen months (July 2011 to December 2012), the SD of each soil layers were approximately represented by the means of what from the corresponding soil layers during the remaining ten months (January 2013 to October 2013). The fractionation values were set to zero for $\delta^{18}\text{O}$ and δD , mainly because there is no isotope fractionation during plant water uptake (Dawson and Ehleringer, 1991; Ehleringer and Dawson, 1992). Model predictions were presented as median (50th percentile) values (Moore and Semmens, 2008), and combined into three source endpoints (0–20, 20–50 and 50–100 cm).

Statistical analyses were performed utilizing the SPSS 17.0 software. One-way analysis of variance (ANOVA) were used to analyze differences of soil water content (SWC), and xylem water $\delta^{18}\text{O}$ and δD among *P. massoniana* ($n=273$), *P. elliotii* ($n=276$) and *C. lanceolata* ($n=165$) at $\alpha=0.05$ level. For detecting the differences of soil water $\delta^{18}\text{O}$ and δD (0–5, 5–10, 10–15, 15–20, 20–30, 30–40, 40–50, 50–60, 60–80 and 80–100 cm, $n=28$ for each soil stratum), multiple comparisons were made using the least significant difference (LSD). The differences between $\delta^{18}\text{O}$ and δD predictions of water source were subjected to Paired t -test.

3. Results

3.1. Seasonal variations of environmental parameters

Multi-year mean monthly AI of 2003–2010 was 0.7 ± 0.2 between July and October, and 2.3 ± 0.2 between November and June (Fig. 1a). Therefore, drought periods were defined from July to October, and the non-drought periods spanned the

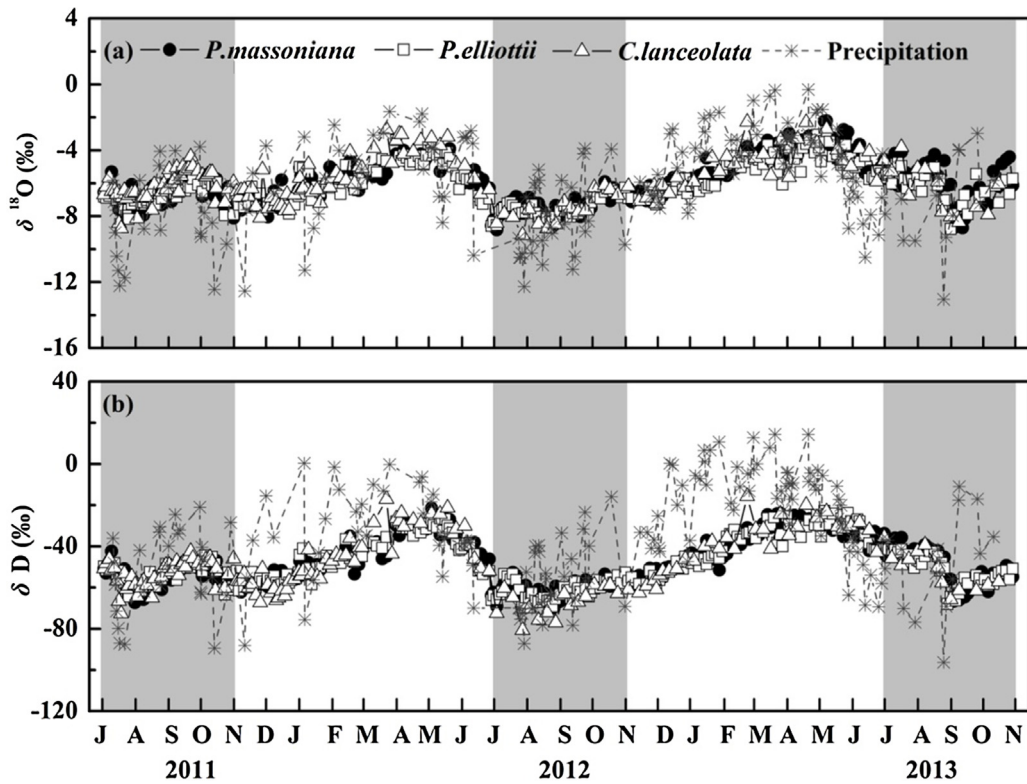


Fig. 2. Seasonal variations of xylem water (a) $\delta^{18}\text{O}$ and (b) δD in *P. massoniana*, *P. elliotii* and *C. lanceolata* between July 2011 and October 2013 at the experimental site. The $\delta^{18}\text{O}$ and δD in precipitation are also presented. Shadow areas indicate the seasonal drought periods (July–October).

remainder months of the year. During the three drought periods of 2011–2012–2013, monthly AI values were 1.1 ± 0.3 , 0.7 ± 0.2 and 0.6 ± 0.4 , which were close to or smaller than one except for October 2011. During the two non-drought periods, monthly AI values were 2.3 ± 0.6 and 1.7 ± 0.4 , respectively. The smaller AI values implied that water deficit was stronger in 2013.

SWC of 5, 20 and 50 cm were generally lower between July and October, and increased from November to June (Fig. 1b). They were all highly variable between the drought and non-drought periods ($p < 0.001$). This trend was just consistent with the seasonal variation of AI. During the drought periods, SWC of 5, 20 and 50 cm were 0.15 ± 0.03 , 0.33 ± 0.03 and $0.38 \pm 0.03 \text{ m}^{-3} \text{ m}^{-3}$, respectively. During the non-drought periods, they increased to 0.23 ± 0.04 , 0.36 ± 0.02 and $0.39 \pm 0.01 \text{ m}^{-3} \text{ m}^{-3}$, respectively.

3.2. Seasonal isotope variations in xylem water

Xylem water $\delta^{18}\text{O}$ and δD did not differ among tree species ($p > 0.05$) during the studied periods (Fig. 2). Therefore, indicating all the trees potentially obtained water from similar depths at the same time. Based on this finding, we would not distinguish trees of *P. massoniana*, *P. elliotii* and *C. lanceolata* in determining the amounts of plant water uptake (see Section 3.4). Values of $\delta^{18}\text{O}$ and δD in precipitation gradually increased from July to April. Then, both $\delta^{18}\text{O}$ and δD declined through May to June. The seasonal variation of $\delta^{18}\text{O}$ and δD in xylem water were almost synchronous with those in precipitation, which mainly reflected the influence of Eastern-Asian monsoon climate (Liu et al., 2008, 2010a,b). Mean $\delta^{18}\text{O}$ and δD of precipitation and xylem water during the drought and non-drought periods are listed in Table 1.

The linear dependence of δD on $\delta^{18}\text{O}$ for tree xylems (*P. massoniana*, *P. elliotii* and *C. lanceolata*) from July 2011 to October 2013 is presented in Fig. 3a. Because the two variables of the regression equation were random and subjected to errors, the

geometric mean regression (GMR) method was used in this study. The local meteoric water line (LMWL: $y = 8.6x + 14.6$, $R^2 = 0.88$, $p < 0.001$) was fitted based on all precipitation data. Relative to the global meter water line (GMWL: $y = 8x + 10$), LMWL had a higher

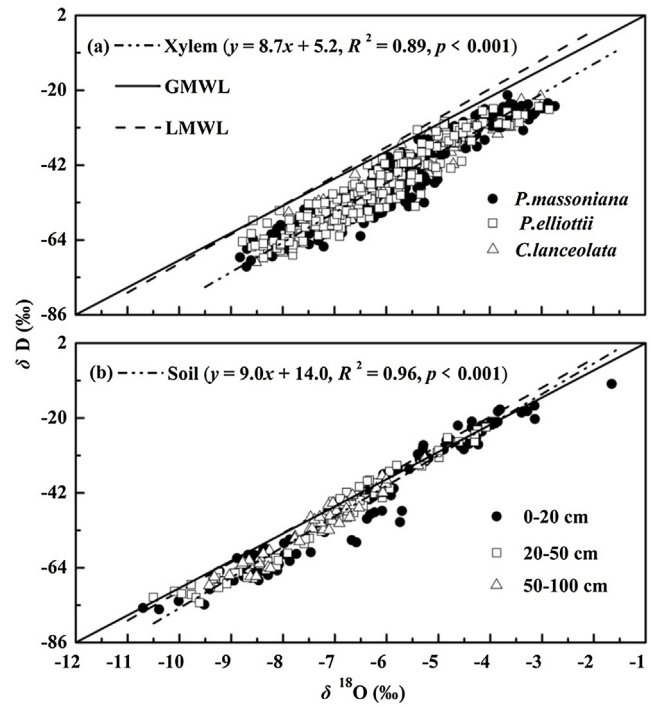


Fig. 3. Values of δD as a function of $\delta^{18}\text{O}$ for (a) tree xylems (*P. massoniana*, *P. elliotii* and *C. lanceolata*) and (b) soil water between July 2011 and October 2013 at the experimental site. The global meter water line (GMWL, $y = 8x + 10$) and local meteoric water line (LMWL, $y = 8.6x + 14.6$) are plotted in each panel for references.

Table 1
Mean $\delta^{18}\text{O}$ and δD of precipitation, xylem and soil water during the drought (July–October) and non-drought (November–June) periods.

	Drought period (July–October)			Non-drought period (November–June)	
	2011	2012	2013	2011–2012	2012–2013
Precipitation $\delta^{18}\text{O}$	-7.78 ± 2.50	-8.29 ± 2.26	-6.88 ± 2.65	-5.39 ± 2.73	-4.52 ± 1.94
Xylem $\delta^{18}\text{O}$	-6.50 ± 0.75	-7.40 ± 0.63	-5.97 ± 1.06	-5.81 ± 1.18	-4.96 ± 1.17
0–20 cm soil $\delta^{18}\text{O}$	-8.06 ± 1.66	-8.23 ± 0.94	-7.94 ± 1.78	-6.91 ± 1.61	-5.16 ± 1.30
20–50 cm soil $\delta^{18}\text{O}$	-8.72 ± 0.58	-8.15 ± 0.82	-7.18 ± 1.06	-7.70 ± 1.66	-5.82 ± 1.02
50–100 cm soil $\delta^{18}\text{O}$	-8.07 ± 0.22	-7.03 ± 0.43	-5.83 ± 0.84	-7.67 ± 0.89	-6.52 ± 1.14
Precipitation δD	-51.8 ± 17.9	-56.1 ± 17.5	-47.5 ± 23.6	-32.0 ± 21.5	-20.3 ± 20.4
Xylem δD	-52.7 ± 5.8	-60.8 ± 4.5	-50.8 ± 8.2	-44.5 ± 11.2	-39.9 ± 10.1
0–20 cm soil δD	-61.4 ± 11.1	-65.9 ± 8.1	-61.9 ± 14.6	-48.5 ± 14.9	-32.4 ± 12.0
20–50 cm soil δD	-65.0 ± 2.6	-66.3 ± 5.0	-53.9 ± 9.3	-54.3 ± 14.0	-37.4 ± 8.7
50–100 cm soil δD	-57.9 ± 2.9	-57.6 ± 6.3	-41.9 ± 7.0	-55.2 ± 8.7	-44.4 ± 9.3

slope and intercept, which could be also seen in other tropical forests (Querejeta et al., 2007). The $\delta^{18}\text{O}$ and δD in xylem water ($y = 8.7x + 5.2$, $R^2 = 0.89$, $p < 0.001$) consistently plotted to the right of LMWL, indicating the utilization of enriched water resources subject to evaporation (Williams and Ehleringer, 2000; Querejeta et al., 2007).

3.3. Seasonal isotope variations in soil water

Soil water $\delta^{18}\text{O}$ and δD underwent pronounced seasonal changes with both depths and months (Fig. 4). There were no statistical difference for the $\delta^{18}\text{O}$ and δD values among depths of the upper 0–20 cm soil stratum (0–5, 5–10, 10–15 and 15–20 cm) ($p > 0.05$), however, which differed markedly from that below 20 cm ($p \leq 0.006$). Furthermore, no significant difference was found among depths of the 20–100 cm soil stratum (20–30, 30–40, 40–50, 50–60, 60–80 and 80–100 cm) for both $\delta^{18}\text{O}$ and δD ($p > 0.05$). Therefore, $\delta^{18}\text{O}$ and δD of 0–100 cm soil stratum were divided into three layers of 0–20, 20–50 and 50–100 cm to represent the shallow, middle and deep soil water, respectively.

Both $\delta^{18}\text{O}$ and δD differed significantly between the drought and non-drought periods for shallow (0–20 cm) ($p < 0.001$) and middle soil water (20–50 cm) ($p \leq 0.002$), but not for deep soil water (50–100 cm) ($p > 0.05$). During the drought periods, values of $\delta^{18}\text{O}$ and δD were more negative in shallow soil (0–20 cm) because of the influences of depleted precipitation (Fig. 2). As the influences of precipitation weakened (Fig. 5), $\delta^{18}\text{O}$ and δD were gradually increased from middle (20–50 cm) to deep soil (50–100 cm). On the contrary, $\delta^{18}\text{O}$ and δD of soil water were generally increased from shallow (0–20 cm) to deep (50–100 cm) stratum during the non-drought periods. Months of September–October and May–June were the “transition periods”, during which soil water $\delta^{18}\text{O}$ and δD gradients within the sample depth were often quite weak. Mean values of $\delta^{18}\text{O}$ and δD in soil water during the drought and non-drought periods are also listed in Table 1.

The co-variation between $\delta^{18}\text{O}$ and δD of soil water is mainly influenced by two independent processes, which are evaporation and mixing between new and old water (i.e. rain). Soil water $\delta^{18}\text{O}$ and δD ($y = 9.0x + 14.0$, $R^2 = 0.96$, $p < 0.001$) plotted to the right of LMWL, indicating the enrichment associated with evaporation (Fig. 3b). Moreover, $\delta^{18}\text{O}$ and δD of 0–20 cm soil water had a steeper slope ($y = 9.6x + 16.1$, $R^2 = 0.96$, $p < 0.001$) compared with the 20–50 cm ($y = 9.4x + 15.8$, $R^2 = 0.91$, $p < 0.001$) and 50–100 cm soil water ($y = 8.9x + 16.7$, $R^2 = 0.85$, $p < 0.001$). This may be due to the greater relative contribution of precipitation to the upper soil layer (Dawson and Simonin, 2011).

3.4. Seasonal variations in depth of water uptake

The depths of water uptake can be estimated by the isotopic intersections between xylem water vertical lines and soil water

stratum (Ehleringer and Dawson, 1992). If there are more than one intersections (e.g., the circumstance of December 2011 in Figs. 6 and 7), the conjunct one (the intersection between 0 and 10 cm) is usually chosen as the main depth of water source (Wang et al., 2010). According to this graphical inference method, the studied tree species derived most of their water from deep soil (50–100 cm) between July and August, and more water from shallow soil (0–20 cm) between November and April. Nevertheless, xylem water and soil water often overlapped at both shallow and deep soils for $\delta^{18}\text{O}$ and δD during the “transition periods”. That means trees might uptake water from shallow or deep soil, or from both layers.

The MixSIR model also predicted that trees tapped predominantly deep soil (50–100 cm) water between July and August of the three drought periods (Fig. 8). The uptake fractions were $54.9 \pm 12.3\%$ and $41.0 \pm 8.6\%$ according to $\delta^{18}\text{O}$ and δD , respectively. Between November and April of the first non-drought periods, both $\delta^{18}\text{O}$ and δD predicted that the main water source for trees shifted to shallow soil (0–20 cm). The percentages of water from this layer accounted $75.8 \pm 12.3\%$ and $60.6 \pm 16.7\%$ according to $\delta^{18}\text{O}$ and δD , respectively. Note that, outcomes estimated by δD were not reliable in December 2011, because there were two intersections of xylem and soil water for δD . Between November and April of the second non-drought periods, water sources partitioning based on $\delta^{18}\text{O}$ and δD showed different water use patterns. Based on $\delta^{18}\text{O}$, trees primarily extracted water from shallow soil (0–20 cm). Based on δD , however, it predicted that trees utilized water of all three depths. Similar to the direct inference method, the model outputs were not reliable during the “transition periods”. We also analyzed these same data using the IsoSource model (Phillips et al., 2005). When the uncertainties in isotope signatures were not incorporated, IsoSource outputs would increase $15.0 \pm 6.3\%$ according to $\delta^{18}\text{O}$ (or $7.1 \pm 5.1\%$ according to δD) of deep soil water utilization during the drought periods, and $7.9 \pm 6.0\%$ according to $\delta^{18}\text{O}$ (or $5.2 \pm 3.9\%$ according to δD) of shallow soil water utilization during the non-drought periods.

4. Discussion

4.1. Comparison of water source partitioning based on dual stable isotopes

We found that the $\delta^{18}\text{O}$ and δD predictions of water source showed similar trends of seasonal variations throughout most of the study periods (Fig. 8). However, water sources predicted by $\delta^{18}\text{O}$ indicated that trees derived more deep soil water (increased $13.8 \pm 3.9\%$ relative to δD) during the drought periods, and more shallow soil water (increased $22.4 \pm 10.5\%$ relative to δD) during the non-drought periods. The differences of plant water source partitioning based on $\delta^{18}\text{O}$ and δD reached the level of statistical significance during both the drought ($p = 0.008$) and non-drought

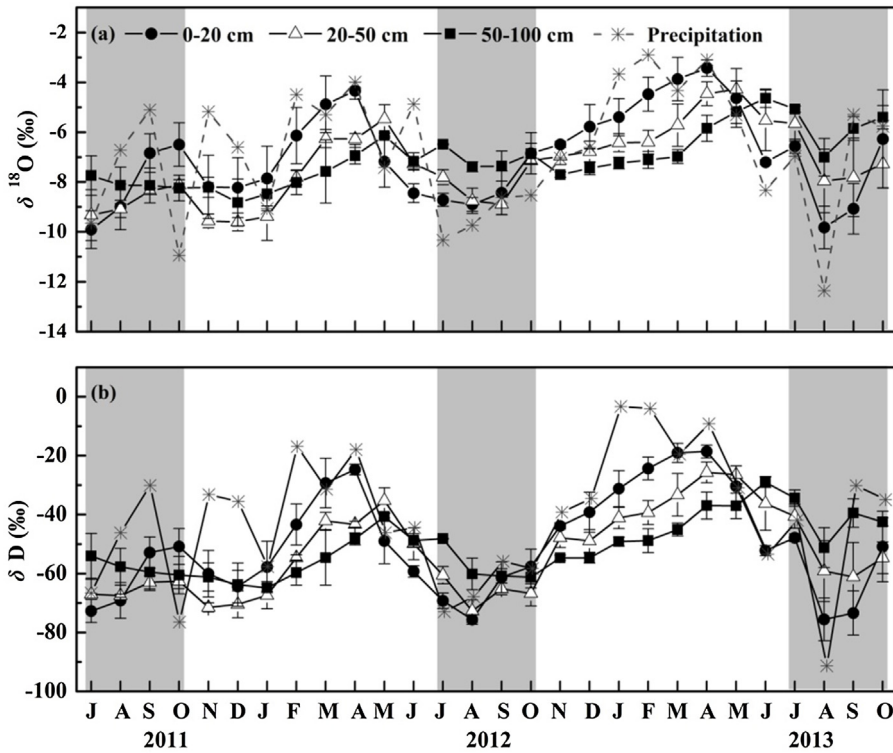


Fig. 4. Seasonal variations of soil water (a) $\delta^{18}\text{O}$ and (b) δD between July 2011 and October 2013 at the experimental site. The $\delta^{18}\text{O}$ and δD in precipitation are also presented. Shadow areas indicate the seasonal drought periods (July–October).

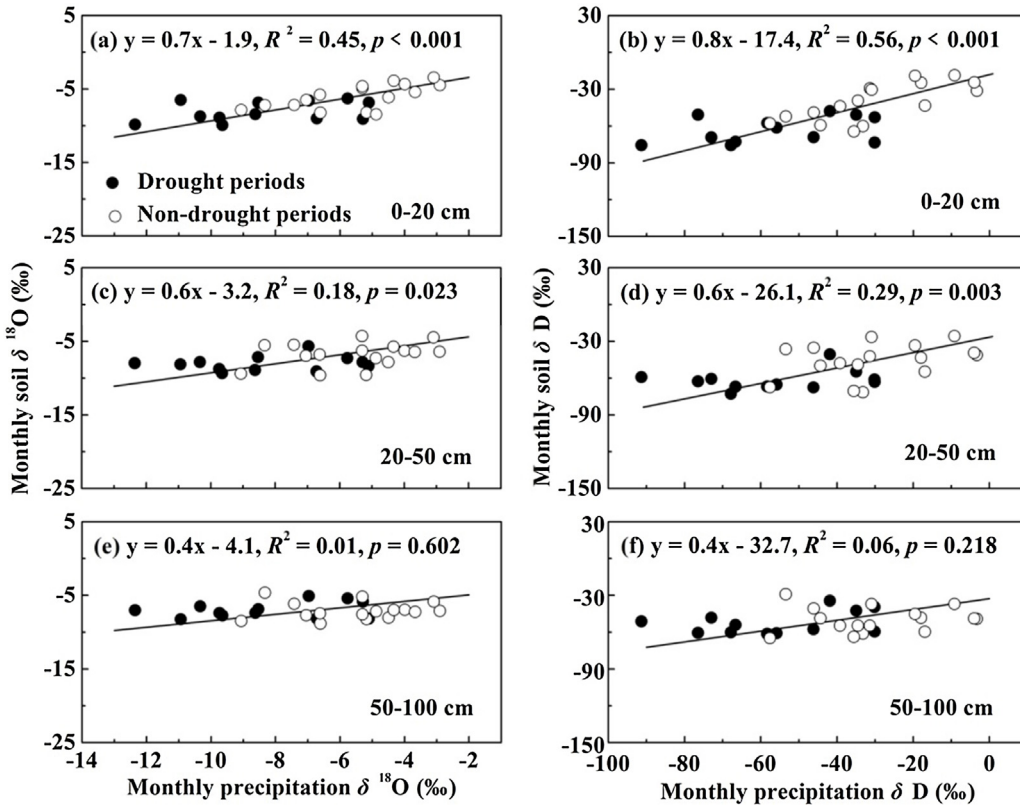


Fig. 5. Relationships between monthly precipitation (weighted means by precipitation amount) and soil water for (a, c and e) $\delta^{18}\text{O}$ and (b, d and f) δD between July 2011 and October 2013 at the experimental site.

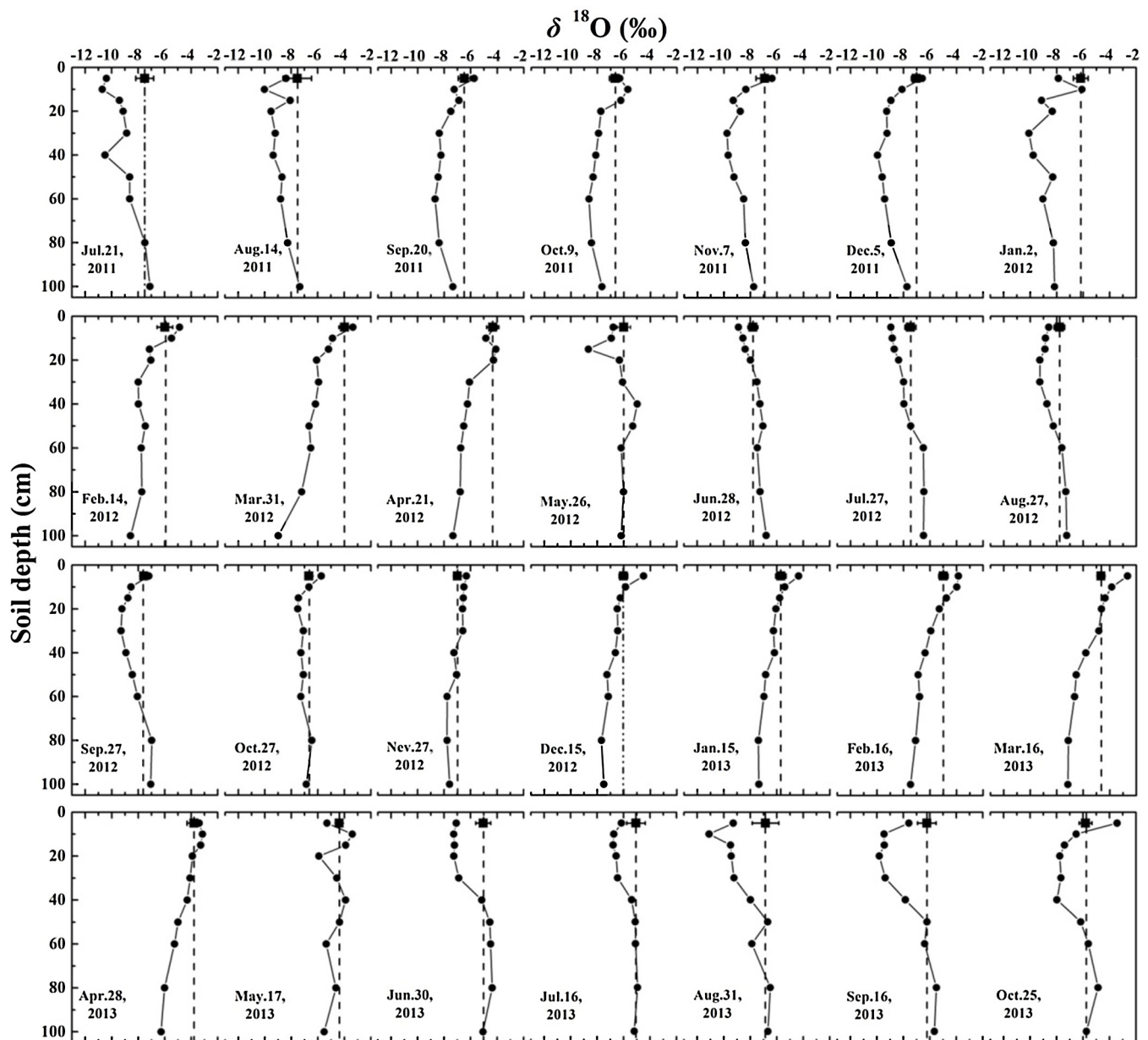


Fig. 6. Seasonal variations of $\delta^{18}\text{O}$ in soil stratum (0–100 cm) and xylem water (mean value of xylem water in *P. massoniana*, *P. elliotii* and *C. lanceolata* \pm SE) between July 2011 and October 2013 at the experimental site.

periods ($p=0.009$). These results can only be brought in line if the isotopic intersections between xylem and soil water shifted farther to the left for $\delta^{18}\text{O}$ (Fig. 6) or to the right for δD (Fig. 7). Most of the previous studies did not discuss the above discrepancy in detail (Cramer et al., 1999; West et al., 2007; Eggenmeyer et al., 2009; Wang et al., 2010; Rossatto et al., 2012), mainly due to the similar graph patterns of isotopic values for $\delta^{18}\text{O}$ and δD . However, the considerable differences similar to our studies were also found in studies of Li et al. (2007), Orłowski et al. (2013) and Meißner et al. (2014). The discrepancy is explained as an artifact of analytical procedure (Meißner et al., 2014) or unknown processes, which could not be explained with their available data (Li et al., 2007; Orłowski et al., 2013).

It seemed unlikely this systematic discrepancy between $\delta^{18}\text{O}$ and δD outcomes in our study was the result of a natural discrimination of $\delta^{18}\text{O}$ and δD in soil stratum. Theoretically, even if the naturally occurring soil stratum of $\delta^{18}\text{O}$ and δD are different

because of isotopic fractionation processes (e.g., evaporation, isotopic exchange reactions with carbonates), the definition from which depths plants really take up water would be still reflected by plant xylem isotopic compositions. This is because plant water uptake is considered as a non-fractionation process (Dawson and Ehleringer, 1991; Ehleringer and Dawson, 1992) besides some halobiotic (Lin et al., 1993) and xerophytic (Ellsworth and Williams, 2007) species. Therefore, this discrepancy was most probably due to an artifact of the post analytical procedure (i.e. cryogenic vacuum distillation and analyses of $\delta^{18}\text{O}$ and δD with an IRIS system).

When the cryogenic vacuum distillation and IRIS methods are applied, it is important to recognize the potential for reduced accuracy in δD analysis as compared to $\delta^{18}\text{O}$. This is partly because it is inability to create an ethanol correction curve for δD (Schultz et al., 2011). In this study, the slightly contaminated ($\text{BB} < 1.2$) xylem water δD were corrected based on our calibration

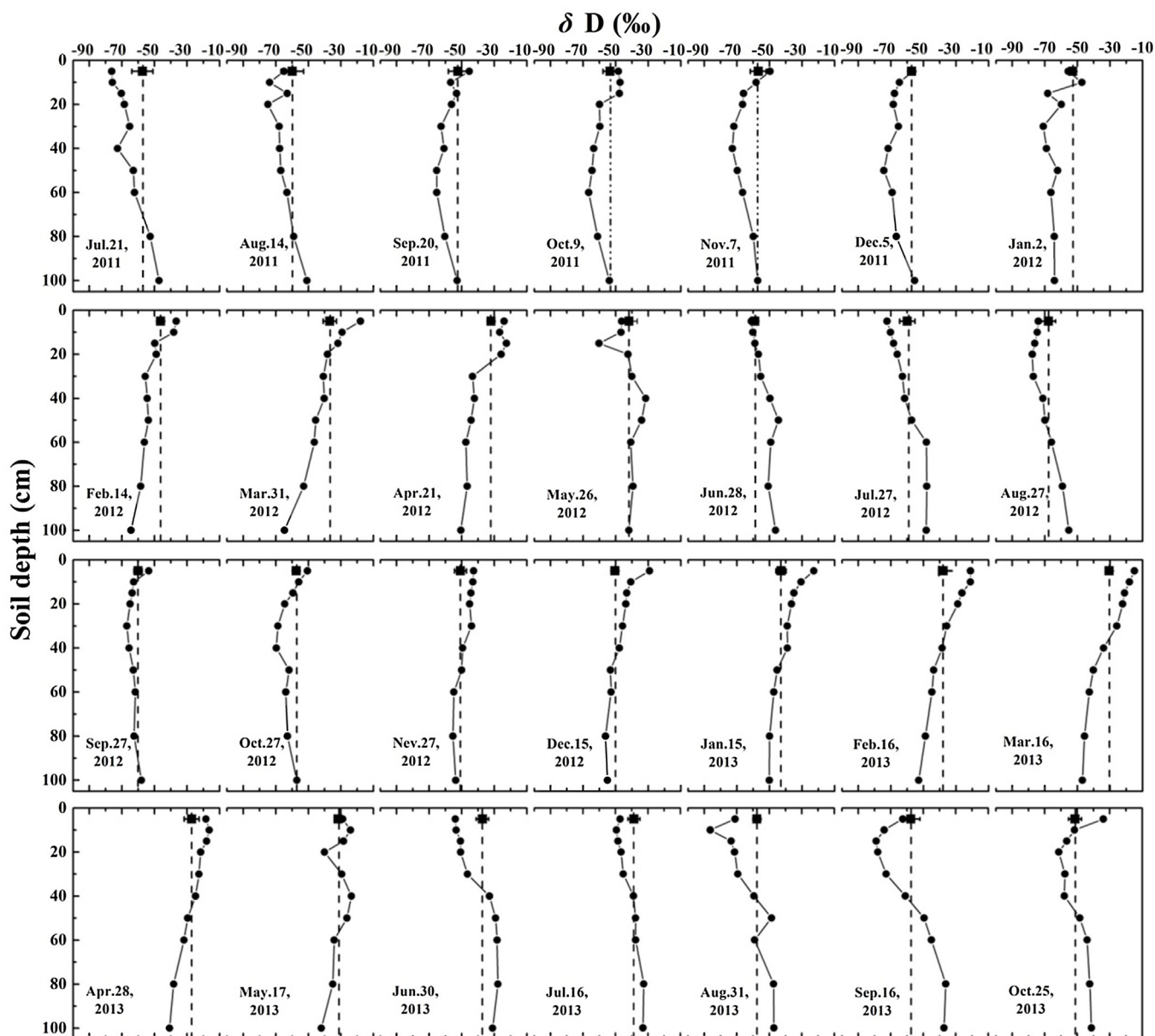


Fig. 7. Seasonal variations of δD in soil stratum (0–100 cm) and xylem water (mean value of xylem water in *P. massoniana*, *P. elliotii* and *C. lanceolata* \pm SE) between July 2011 and October 2013 at the experimental site.

curves (Xiao et al., 2012). However, the correction curve for δD ($y = -0.23x + 1.09$, $R^2 = 0.88$, $p = 0.04$) was not as robust as that for $\delta^{18}O$ ($y = -0.15x + 0.99$, $R^2 = 0.99$, $p < 0.001$). A sensitivity analysis showed that the discrepancy of $\delta^{18}O$ and δD predictions would disappear during the drought periods ($p = 0.85$), when the uncorrected xylem water δD were applied (this would increase δD to higher values). The discrepancy was also decreased during the non-drought periods, but it still existed ($p = 0.022$). Thus, the δD in xylem water might have been, to some degree, over-compensated.

A recent study reported that isotopic exchanges between $\delta^{18}O$ and δD of soil water and carbonates (e.g., calcium carbonate) can react in a low field temperature (Zeebe, 2009). As mentioned above, this equilibrium fractionation in field will not influence the definition of plant water source. However, Meißner et al. (2014) indicated that although the exchange reactions still exist, it will be degraded during the cryogenic vacuum distillation at a higher temperature ($\sim 110^\circ C$). They showed that soil carbonates decrease the $\delta^{18}O$ of extracted soil water ($\sim 1.3\%$), but the δD is

not markedly altered. Additionally, because their water isotopic compositions were determined by IRMS (Isotope Ratio Mass Spectrometry) method, the influences of spectral contamination on $\delta^{18}O$ and δD could be eliminated (Schultz et al., 2011). If soil water $\delta^{18}O$ of our study were also affected by the soil carbonates, the vertical soil stratum of $\delta^{18}O$ should be shifted to right, that is to say, the isotopic intersections should be shifted to left. A sensitivity analysis showed that the different outcomes from $\delta^{18}O$ and δD predictions were not significant during both the drought ($p = 0.073$) and non-drought periods ($p = 0.628$) when we increased the $\delta^{18}O$ values of different soil layers with 0.9‰.

Furthermore, soil clay content (West et al., 2006; Orłowski et al., 2013; Meißner et al., 2014) and soil water content (Meißner et al., 2014) may also bias the $\delta^{18}O$ and δD values of extracted soil water. However, these soil physicochemical properties influence both the dual stable isotopes (Meißner et al., 2014), and could not be the main reasons for the discrepancy in our study. Thus, we should consider the influences of artifacts in determining plant water sources,

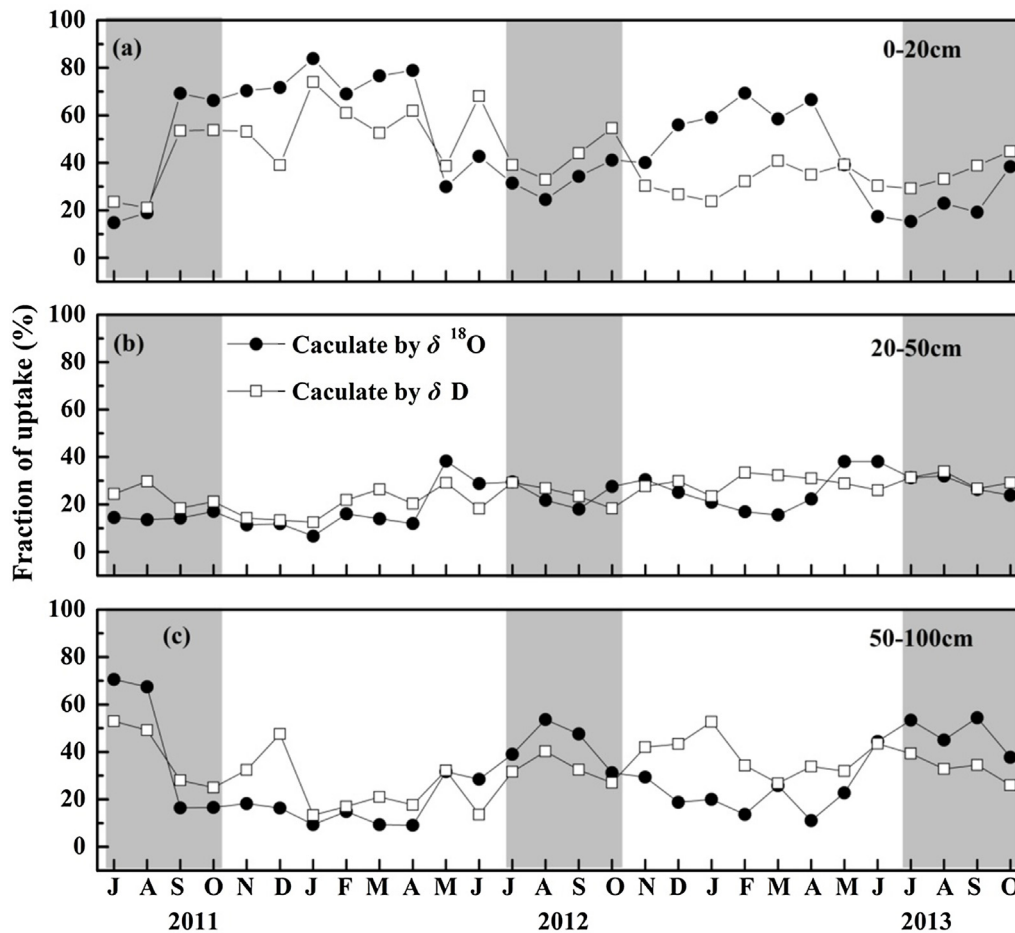


Fig. 8. Seasonal variations of water uptake from (a) shallow soil (0–20 cm), (b) middle soil (20–50 cm) and (c) deep soil (50–100 cm) for trees (*P. massoniana*, *P. elliotii* and *C. lanceolata*) based on MixSIR between July 2011 and October 2013 at the experimental site. Shadow areas indicate the seasonal drought periods (July–October).

especially when just one of the dual stable isotopes of $\delta^{18}\text{O}$ and δD was applied.

4.2. Effects of seasonal drought on water uptake for trees

Our results showed that tree species of *P. massoniana*, *P. elliotii* and *C. lanceolata* exhibited strong plasticity in sources of water uptake (Figs. 6–8). This result was consistent with the findings of several other studies, which reported that trees could shift water sources between deep and shallow soil water (McCole and Stern, 2007; Eggemeyer et al., 2009; Liu et al., 2010a,b). However, xylem water $\delta^{18}\text{O}$ and δD did not differ among tree species (*P. massoniana*, *P. elliotii* and *C. lanceolata*) during the studied periods (Fig. 2), indicating that the studied trees competed for water resources from similar depths. This finding contrasts that of West et al. (2007) and Eggemeyer et al. (2009), who demonstrated that coexisting woody species would adopt different water use strategies in water-limited environments. In our site, three evergreen coniferous tree species were planted around the same period of 1985, which had very similar heights and stem diameters (see Section 2.1). A previous study at this site also suggested that vertical root distributions of the above trees are quite similar (Tang et al., 2014a). Taking these aspects into consideration, we probably could understand that different tree species of this plantation might adopt similar traits to maintain a high resistance to drought stress, such as defoliation and changing water sources from shallow to deeper soil water.

According to both the graphical inference and MixSIR methods, the studied trees extracted water predominantly from deep

soil (50–100 cm) between July and August, and from shallow soil (0–20 cm) between November and April. However, water sources in the remaining months (September to October, and May to June) could not be determined exactly, due to the weak gradients of $\delta^{18}\text{O}$ and δD in soil stratum. At this point, water source should be determined by considering other environmental parameters, such as soil water content (Wang et al., 2010), leaf water potential (Stahl et al., 2013) and through-fall precipitation (Romero-Saltos et al., 2005), etc. During the drought periods, the heavy reliance on water of deep soil (50–100 cm) suggested that surface water (0–50 cm) was becoming insufficient for trees between July and August. As SWC of 0–50 cm were still relatively lower between September and October (Fig. 1b), it was possible to infer that trees were still absorbing water from deep soil (50–100 cm). From November to April, SWC of 0–50 cm increased gradually, and trees appeared to acquire water preferentially from shallow soil (0–20 cm). Because SWC of 0–50 cm were still ample during May and June, we could infer that the major water sources for trees probably remained shallow soil water.

When precipitation recharged soil water was not available during the drought periods, the shifting of water utilizations from shallow to deep soil might play an important role in maintaining long-term survival for the plantations, especially when different tree species of this plantation showed inter-specific competition for similar water resources. Because the vertical root distributions may not be a reliable indicator of actual water uptake dynamics in either time or space (Ehleringer and Dawson, 1992). This pattern of water utilization, apparently, could not be learned from the former

roots excavation. Other studies conducted in seasonally dry tropical (Liu et al., 2010a,b) and subtropical (Nie et al., 2011) ecosystems have also demonstrated that trees are functionally dimorphically rooted. Those trees included both deciduous (e.g., *Radermachera sinica* Hemst. and *Pometia tomentosa* (Bl.) Teysm. et Binn.) and evergreen (e.g., *Girardinia subaequalis* Planch.) species.

We should also note that a time lag effect typically exists between the starting of a water shortage and the identification of its consequences (McDowell et al., 2008; Vicente-Serrano et al., 2013; Tang et al., 2014a). That is to say, a faster water depletion occurred at the beginning of drought periods might have an influence on the subsequent plant water uptake and physiological activities. For example, Tang et al. (2014a) indicated that there is a 19-days time lag between stomatal closure and leaf senescence during the drought periods in this plantation. However, we could not provide enough information to reflect the relationship between tree water source shifting and the physiological activities by the monthly sampling data. Furthermore, there is mounting evidence that hydraulic redistribution (HR) frequently occurs in ecosystems containing woody plants with dimorphic root systems (Baker and Vanbavel, 1986; Bleby et al., 2010; Neumann and Cardon, 2012). These studies have shown that HR may also moisten surface soil, facilitate nutrient acquisition and delay the onset of soil drying during the drought periods (Bleby et al., 2010; Neumann and Cardon, 2012). Further studies on high temporal resolution of plant water source partitioning can be expected to provide more insights to the above-mentioned issues.

5. Conclusions

In this study, we applied the dual stable isotopes of $\delta^{18}\text{O}$ and δD to detect the impacts of seasonal drought on plant water sources. Our results indicated that xylem water $\delta^{18}\text{O}$ and δD did not differ among tree species of *P. massoniana*, *P. elliotii* and *C. lanceolata*, suggesting that they competed for water resources from similar depths during both the drought and non-drought periods. Both $\delta^{18}\text{O}$ and δD predicted that trees mainly derived water from shallow soil during the non-drought periods, and could shift to tap deep soil as the drought periods progressed. The $\delta^{18}\text{O}$ predictions, however, showed that trees derived more $13.8 \pm 3.9\%$ water (relative to δD) from deep soil during the drought periods and more $22.4 \pm 10.5\%$ water (relative to δD) from shallow soil during the non-drought periods. These discrepancies probably resulted from the artifacts of cryogenic vacuum distillation and analyses of $\delta^{18}\text{O}$ and δD with the IRIS system.

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