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# Isotopic analysis of water sources of mountainous plant uptake in a karst plateau of southwest China

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## Abstract:

Ecosystem in the karst region of southwest China is very fragile due to a very limited amount of water storage for plant uptake in the thin and rocky soils underlain by rock fractures. Plants in these karst regions are thought to take water from the soils and shallow fractured rock zone (subcutaneous zone) as well. However, the role of subcutaneous water in maintaining karst vegetation remains unclear, and proportions of the water sources for plant uptake in different environment conditions are unknown. In this study, five typical species of plants at two sites were selected in a karst plateau of Qingzhen, central Guizhou Province of China. Proportions of the possible water sources contributed for the plant uptake from two soil layers and subcutaneous zone were determined on the basis of $\delta$D and $\delta^{18}$O values of plant stem water, soil water and subcutaneous water. The analysis reveals that most plants take water from the soil layers and the subcutaneous zone as well, but proportions of these water contributions for plant uptake vary seasonally and depend on site-specific conditions and plant species. Plant uptake of the subcutaneous water for all species averages less than 30% of the total monthly amount in June and September, compared with more than 60% in dry December. Plants tend to take a larger proportion of water from the upper soil layer at the bush site than at the forest site in June and September (63 *vs* 28% in July; 66 *vs* 54% in September for all species in average). In December, however, 98% of water is taken from the subcutaneous zone at the bush site which is much greater than 68% at the forest site. Compared to deciduous arbor, evergreen shrub takes a greater proportion of subcutaneous water in the December drought. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS stable isotopes; mountainous plant; soil water; subcutaneous water

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## INTRODUCTION

Because infiltrated water for plant uptake stores in both the heterogeneous soils and rock fractures, identifying water sources for plant uptake in the karst region is a very challenging task for the botanists and hydrologists, compared to a non-karst region. Root distribution pattern is regarded as a primary factor for tracing water sources of plant uptake. Root distribution is associated with its surrounding environment and controls plant growth for xylophyta. For example, rooting depth in the tropic region is negatively correlated with annual precipitation but positively correlated with the length of the drought season (Schenk and Jackson, 2002). The species of karst plants are able to survive in serious drought periods, showing that some species of plants might obtain a stable water source through extension of plant roots (Benjamin *et al.*, 2001). Distinguishing the root system, especially in karst regions, is very arduous work and data on root distribution are rarely available (Schenk and Jackson, 2005) because of a strong heterogeneity of soil thickness and composition and unpredictable fracture patterns in the limestone below the soils. The non-continuous shallow soil and the unique crannies, rock apertures and grooves of consolidated rocks result in significantly different patterns of the root distribution. Ironically, more roots do not mean a more effective in plan uptake of water even though roots are the plant's organ to absorb soil moisture (Flanagan *et al.*, 1992; Thorburn and Walker, 1994). Field measurement of root distribution can only provide a little information about absolute rates and/or patterns of water resource utilization (Ehleringer and Dawson, 1992).

Analysis of $\delta$D and $\delta^{18}$O at different soil layers is an effective way for tracing water sources for plant uptake because of no isotopic fractionation during terrestrial plant uptake of water (Wershaw *et al.*, 1966). The hydrogen isotope content of xylem sap that does not expose it to evaporative enrichment reflects the water sources consumed by plants. Although all the water sources ultimately come from precipitation, the groundwater isotopic composition reflects broader, long-term precipitation or water management patterns, while soil water represents mostly short-term precipitation at a given site (Ewe *et al.*, 1999). Shallower soil water is generally more enriched in heavier isotopes compared to the deeper soil water due to evaporation (Sternberg *et al.*, 1991). Therefore, values of $\delta$D and $\delta^{18}$O for various water sources are significantly different because of evapotranspiration. If the hydrogen and oxygen stable isotope ratios of potential

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water sources for plant uptake are significantly different, stable isotope composition of water extracted by plant stems can be used to quantitatively determine the relative contributions of the different water sources (Thorburn and Walker, 1993). Stable isotope composition of water has been used to trace water uptake patterns for many plant communities, such as mountain forest (Ish-Shalom *et al*., 1992; Ewe *et al*., 1999; Atsuko *et al*., 2002; Peñuelas and Filella, 2003), riparian forest (Dawson and Ehleringer, 1991; Smith *et al*., 1991; Busch *et al*., 1992; Thorburn and Walker, 1993; Mensforth *et al*., 1994; Dawson and Pate, 1996; Jolly and Walker, 1996), maritime forest (Valentini *et al*., 1995; Slavich *et al*., 1999), desert plants and cropland (Smith *et al*., 1997; Sekiya and Yano, 2002), and karst plants rooted within weathered zone (Querejeta *et al*., 2006, 2007; McCole and Stern, 2007). However, quantitative analysis of contributions of water sources for plant uptake in the rather heterogeneous karst region of southwest China is not as reported, and influences of the site-specific environments and ecotype on water uptake for various plant species are unknown.

The karst plateau-gorge area is located in the subtropical humid monsoon regions of southwest China. Soil in the karst area is usually thin (less than 50 cm in average) and unevenly distributed. The shallow soil underlain by abundant rock fractures leads to a large percolation capacity but weak water retaining capacity in the soil zone. An unevenly distributed rainfall pattern in this karst area (70% of rainfall concentrated during the rainfall season of April to September) makes a very limited amount of water in the soil zone for plant uptake. This condition may severally inhibit plant growth in this region, especially in the drought period.

In the karst region of southwest China, the bedrock is constituted primarily of pure carbonate with an old stratum deposited in the Triassic period, and it is characterized by dense structure, low porosity (<3%) and low hydrochloric acid (HCl) insoluble matter (<4%) (Yuan, 1994). However, the upper part of the bedrock is usually weathered and has rich fractures. This weathered zone, called as a subcutaneous zone, may form a permanently saturated zone (Williams, 1985) and thus becomes a water source for plant uptake.

In this study, we investigated three potential water sources available for plant uptake: rainwater captured by two soil layers of the upper 10 and lower 10–50 cm and the underlying subcutaneous water at a karst catchment in the uplands of the Qingzhen Plateau, Guizhou Province of China. We analyzed $\delta$D and $\delta^{18}$O values of the five dominant plant species at the study catchment to quantify proportions of water uptake by plants from the three water sources in two different environments of the relic forest patch and the bush patch which is degenerated from forest. We used changes in predawn water potential (PWP) from wet to dry season to assess water stress of two plant communities of arbor and shrub, which provide additional information about plant uptake capacity from the three water sources. This analysis helps us understand the competition and utilization strategies for plant uptake of water in the karst region. The results are useful for ecosystem management in the ecological degradation area of southwest China.

## SITE DESCRIPTION

The study site, Wangjiazhai catchment (106°20′5″ ~ 106°21′8″E, 26°31′45″ ~ 26°30′27″N) is located in the Qingzhen Plateau of the central Guizhou Province, southwest China (Figure 1). It covers an area of 2·4 km$^2$ and the landscape is dominated by a typical karst peak-cluster depression in the karst region of southwest China. Ground surface elevation varies from 1275 m in the flat parts to 1451 m in the small mesas. Soils mixed with pure limestone and dolomite are sparsely distributed. The area with exposed rocks accounts for about 70%. The largest thickness of soil at the observed sites is 40–60 cm. Subsurface runoff generated in the fractured zone (subcutaneous zone) discharges from several springs at the lower areas of hillslopes.

Climate in the study area has a distinct seasonal variation of the humid subtropical monsoon zone. Yearly average rainfall is 1205·6 mm, with a rainfall season from April to September and a dry season from November to March. During the study period of 1 November 2005 to 31 October 2006, the total precipitation was 721 mm, about 70% of the total amount in a normal year. Rainfall amount during May to September was 468·7 mm, about 65% of the yearly total amount (Figure 2) according to the records of a rainfall gauge within the study site.

Forest and bush appear mostly on the hillslopes with abundant cracks, fissures, channels and small solution holes covered by soils (Figure 1). In the relic forest patches, the plant community has a similar canopy of arbors (*Platycarya longipes, Carpinus pubescens,* etc.) with an understory of shrub–herb consisting of *Rhamnus*

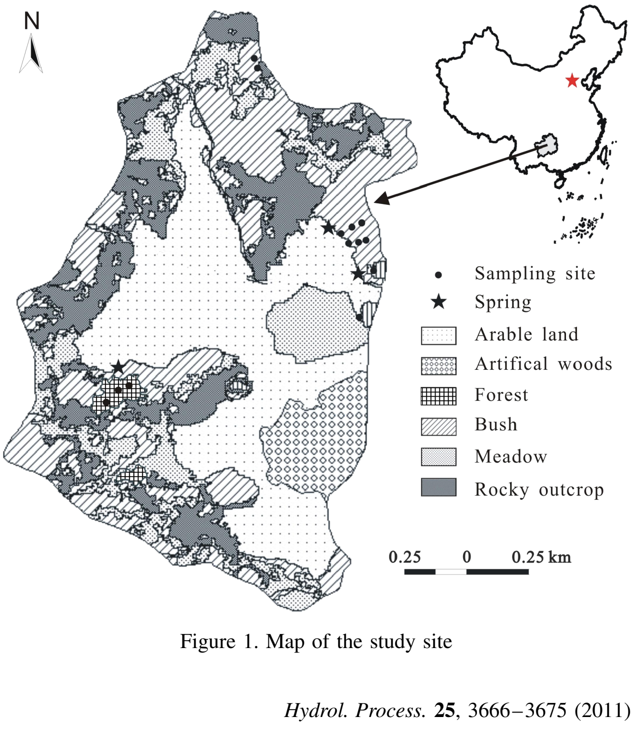

Figure 1. Map of the study site

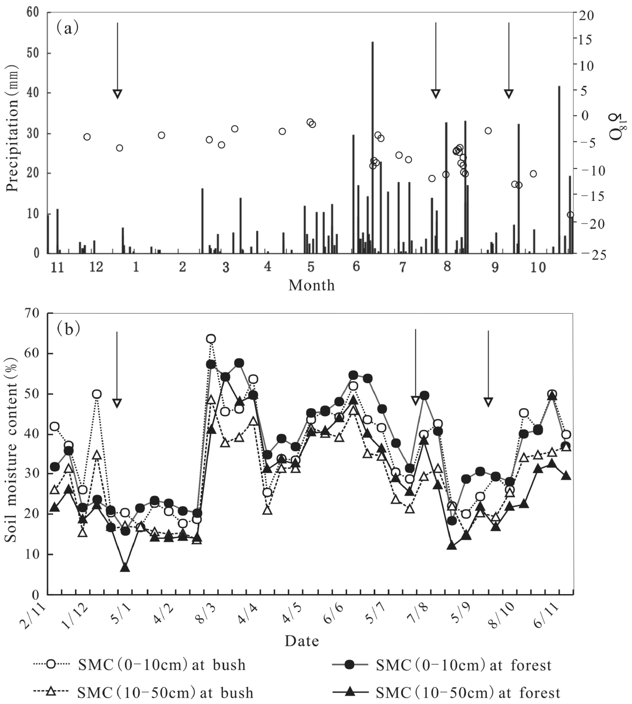

Figure 2. Monthly variation of precipitation, and $\delta^{18}O$ values of rainfall samples (a), and soil moisture content (SMC) at the bush and forest sites (b) during November 2005 to December 2006. The three arrows indicate dates on which isotope samples of soil water, subcutaneous water and stem water were collected and analyzed

*davurica, Zanthoxylum planispinum, Viburnum utile*, etc. Hierarchy of arbor community stratum has no significant differences in phytocenosium structure. The height of arbor is usually low and most of its species do not reach their biological heights because the available water stored in the karst soil is limited for plant uptake. The shrubs of *Rosa cymosa, Z. planispinum, Pyracantha fortuneana, V. utile, Rubus biflorus*, etc. are grown in the ecosystem degeneration areas, e.g. the bush site, as a result of aggressive interference by the human activities of long-term predatory farming. Herbs of *Ficus tikoua, Parathelypteris glanduligera* and *Carex scaposa* occur sporadically. The plant is over 10-years old at the forest site and 2–5-years old at the bush site. For the species of arbor, the heights are 6–12 and 0·8–3 m at the forest and bush sites, respectively; the stem diameters are 5–12 and 1–3 cm, respectively. For the species of shrub, the heights at the forest and bush sites are similar for the most species, e.g. 1–2·4 and 0·8–2·6 m for *V. utile* at the forest and bush sites, respectively, and 0·6–1·2 m for *R. davurica* at both sites, except for a 2-m higher at the forest than at the bush sites for *P. fortuneana* (2–5 *vs* 0·6–2·3 m).

The unconsolidated soils have been derived from limestone through weathering process, contain scattered rock fragments, and have an average thickness of 50 cm. The soil is in yellow color at the study sites and consists of 40–50% clay, 30–45% silt and 5–10% sand, which widely spreads in southwest China and is classified as alumi-haplic acrisol in terms of FAO-Unesco (1988). The average of soil bulk density over the depth interval of 20 cm is 1·35 g/cm$^3$ at the bush site and 1·07 g/cm$^3$ at the forest site (Du and Wang, 2008). Because about 70% of root product is within a 30-cm depth of soil below the ground surface in the southwest karst region (Xiang *et al.*, 2004), water storage in the thin soil is a basic resource for plant uptake.

## SAMPLING AND STABLE ISOTOPE ANALYSES

### *Sampling of water, soil and plants*

Precipitation was recorded for each rainfall event and soil moisture contents at two sites were measured in a time interval of 10 days from 1 November 2005 to 6 November 2006. Samples of plant stem water, soil water at the depth of 10 and 10–50 cm and spring water (subcutaneous water) were collected in the drought season of 20 December 2005 and in the rainfall season of 29 July 2006 and 17 September 2006 (Figure 2).

Sampling of soils and plant species was conducted from 3 to 6 plots at the forest site and the bush site, respectively (Figure 1). The area of each plot is 10 m × 10 m. At each plot, three soil sampling points and three to five plant species were selected for testing their variations. At each sampling point, six soil samples were collected using the hand probes at the depths of 0–5, 5–10, 10–20, 20–30, 30–40 and 40–50 cm below ground. The total soil samples during every measurement period were 54 and 108 at the forest and bush sites, respectively. The soil moisture contents were measured by the gravimetric method, and then averaged to represent the upper layer (0–10 cm) and lower layer (10–50 cm) of soils (Figure 2). Investigation by Du and Wang (2008) demonstrated that variations of annual mean soil moisture contents among the measurement positions at either forest or bush site were not significantly different at the significant level of 0·95.

Five typical species of the native canopy and understory plants were selected in this investigation. They include three shrubs of *V. utile, P. fortuneana* and *R. davurica* and two arbors of *P. longipes* and *C. pubescens*, which appear at two sites of the karst bush patches and relic forest patches. For each plant species, three to five individuals with similar diameter of breast height were selected. The total samples were 25 and 28 for the species of arbor and shrub, respectively, at the forest site, and 26 and 32 for the two species, respectively, at the bush site.

Sampling of twig xylem water was conducted at mid-morning (10 : 00 am) when the plants started transpiration. Fully suberized stems of several centimeters from the shoot tip without leaves were taken for avoiding influence on water sources from the leaves. The stems are 0·5–1·0 cm in diameter and 8–15 cm in length.

A cryogenic vacuum distillation method was adopted for extracting water from plant stem and soil samples (Ehleringer *et al.*, 1989; Dawson and Ehleringer, 1991). Each sample was about 30 g in weight, and was well mixed and immediately sealed into the plastic vials

Table I. Mean and standard deviation (in parentheses) of $\delta$D and $\delta^{18}$O contents of stem xylem, soil water and subcutaneous water

| Vegetation types | Water sources | July 2006 | | September 2006 | | December 2005 | | Average | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\delta$D | $\delta^{18}$O | $\delta$D | $\delta^{18}$O | $\delta$D | $\delta^{18}$O | $\delta$D | $\delta^{18}$O |
| Forest | Subcutaneous flow | −47·38 | −6·03 | −48·23 | −6·54 | −41·55 | −5·53 | −45·72 | −6·03 |
| Bush | | −43·50 | −6·24 | −45·55 | −6·75 | −42·76 | −6·42 | −43·94 | −6·47 |
| Forest | Soil (0–10 cm) | −79·26 | −10·75 | −90·07 | −12·68 | −42·90 | −5·00 | −70·74 | −9·48 |
| | | (3·58) | (0·44) | (1·82) | (1·02) | (1·26) | (0·65) | | |
| | Soil (>10 cm) | −89·20 | −11·20 | −97·28 | −13·39 | −53·40 | −7·35 | −79·96 | −10·65 |
| | | (4·01) | (0·55) | (6·33) | (0·82) | (2·80) | (0·78) | | |
| Bush | Soil (0–10 cm) | −69·81 | −8·86 | −81·24 | −9·48 | −47·78 | −6·74 | −66·28 | −8·36 |
| | | (6·09) | (0·51) | (5·32) | (1·10) | (3·89) | (0·78) | | |
| | Soil (>10 cm) | −77·79 | −9·48 | −84·98 | −10·41 | −61·78 | −8·07 | −74·85 | −9·32 |
| | | (1·73) | (0·22) | (5·91) | (0·70) | (5·68) | (0·57) | | |
| Vegetation types | Species (functional groups) | $\delta$D | $\delta^{18}$O | $\delta$D | $\delta^{18}$O | $\delta$D | $\delta^{18}$O | | |
| Forest | V. utile (Evergreen/shrub) | −73·12 | −8·22 | −82·30 | −9·58 | −38·13 | −4·48 | −64·52 | −7·43 |
| | | (5·08) | (0·28) | (3·63) | (0·33) | (4·14) | (0·22) | | |
| Bush | | −68·32 | −8·29 | −76·05 | −10·33 | −29·01 | −3·32 | −57·79 | −7·31 |
| | | (4·30) | (0·49) | (3·29) | (0·26) | (4·90) | (0·43) | | |
| Forest | P. fortuneana (Evergreen/shrub) | −70·81 | −8·11 | −70·80 | −9·25 | −43·89 | −6·25 | −61·83 | −7·87 |
| | | (1·38) | (0·48) | (2·89) | (0·37) | (1·44) | (0·46) | | |
| Bush | | −69·79 | −8·80 | −76·57 | −8·54 | −33·16 | −3·82 | −59·84 | −7·05 |
| | | (5·99) | (1·67) | (8·51) | (0·98) | (5·78) | (0·75) | | |
| Forest | R. davurica (Deciduous/shrub) | −71·45 | −8·59 | −88·79 | −11·02 | −43·76 | −6·14 | −68·00 | −8·58 |
| | | (2·63) | (0·62) | (4·95) | (1·13) | (6·92) | (1·19) | | |
| Bush | | −70·34 | −8·96 | −78·70 | −9·77 | −31·70 | −3·91 | −60·25 | −7·55 |
| | | (7·98) | (2·04) | (7·01) | (0·62) | (4·44) | (0·26) | | |
| Forest | P. longipes (Deciduous/arbor) | −77·16 | −8·99 | −88·07 | −10·98 | | | −82·62 | −9·99 |
| | | (1·17) | (0·84) | (2·53) | (1·24) | | | | |
| Bush | | −67·86 | −8·55 | −66·67 | −7·73 | −41·85 | −5·73 | −58·79 | −7·34 |
| | | (0·74) | (0·28) | (3·43) | (0·74) | (5·74) | (1·51) | | |
| Forest | C. pubescens (Deciduous/arbor) | −75·35 | −9·28 | −85·36 | −9·84 | −49·48 | −6·57 | −70·06 | −8·56 |
| | | (5·56) | (0·51) | (1·77) | (0·07) | (3·98) | (0·24) | | |
| Bush | | −65·86 | −8·32 | −65·94 | −7·35 | | | −65·90 | −7·84 |
| | | (5·70) | (0·55) | (2·93) | (0·26) | | | | |
| Forest | Average | −73·58 | −8·64 | −83·06 | −10·13 | −43·82 | −5·86 | −69·41 | −8·49 |
| Bush | | −68·43 | −8·58 | −72·79 | −8·74 | −33·93 | −4·20 | −60·51 | −7·42 |

in order to avoid evapotranspiration (Bollard, 1960). Rainfall was collected in 11 polypropylene bottles from the weather station in the study area. Three samples of spring water, one at the forest site and two at the bush site (Figure 1), were collected to analyze isotopic compositions of subcutaneous water. A small quantity of mineral oil was added to each bottle to prevent evaporation (Peñuelas and Filella, 2003).

Water potential of plants in the early morning (3 : 00–6 : 00 am) was measured using a pressure chamber (PSΨPRO, Wescor Inc., USA). PWPs reflect an equilibrium state between plant water and soil moisture content (Larcher, 1997; Snyder and Williams, 2000).

### *Stable isotope analyses*

$\delta^{18}$O of extracted water was analyzed using the standard $CO_2$–$H_2O$ equilibration method (Epstein and Mayeda, 1953; Socki *et al.*, 1992) and $\delta$D was analyzed by the $H_2$–$H_2O$ equilibration technique. All samples of $\delta$D and $\delta^{18}$O were analyzed using equipment of continuous flow MS (Isoprime-GC) at the State Key Laboratory of Environmental Geochemistry, Chinese Academy of Sciences, China. For natural abundance work, the stable isotope composition of a particular material or substance as a ratio relative to an internationally accepted reference standard (Table I) was expressed as:

$$\delta/‰ = [(R_{\text{sample}} - R_{\text{standard}}) - 1] \times 1000 \qquad (1)$$

where the subscript sample is the abundance ratio of those isotopes either the D/H or $^{18}$O/$^{16}$O of the sample, $R_{\text{standard}}$ either the D/H or $^{18}$O/$^{16}$O ratio of standard mean ocean water. For all samples extracting from plants, soils and subcutaneous layers, the analyses precisions of $\delta$D and $\delta^{18}$O (including sampling, extraction and analytical errors) were estimated to be ±4‰ for $\delta$D and ±0·15‰ for $\delta^{18}$O.

### *Mixing models for determination of water sources by plant uptake*

As roots do not fractionate water during plant uptake, evaporation from suberized stems can be negligible. Thus, the isotope ratios of water in plant stems reflect the uptake-weighted average isotope ratio of water in the root zone (Ehleringer and Dawson, 1992), and can be used to differentiate the water sources for plant uptake.

Brunel *et al.* (1995) developed a two soil-layer model for computation of water extraction from each soil layer when the root zone water and twig xylem water isotopic were measured. This model can be extended to three soil-layer situations (Cramer *et al.*, 1999), such as water sources from the upper (<10 cm) and lower (>10 cm) soil layers and form the subcutaneous layer in this study, provided that both $^2$H and $^{18}$O are measured in each layer.

The proportion of water tapped from each layer was assessed quantitatively at each sampling time using the following relationship between tree xylem water $\delta$-values and water extraction from the three water sources.

$$\delta X_{\mathrm{t}} = f_1 \delta X_1 + f_2 \delta X_2 + f_3 \delta X_3 \tag{2}$$

where $X_t$ refers to either the D or $^{18}$O isotope of xylem water of tree stem, $f$ the coefficient of water uptake from each layer ($0 \le f \le 1$), and the subscripts 1, 2 and 3 the layer numbers. The total proportion of water uptake by the plants from the three layers should be equal to 1:

$$f_3 = 1 - f_1 - f_2 \tag{3}$$

The values of $f_1$ and $f_2$ were determined using the linear programming optimization technique for minimizing $R$:

$$R = (\delta \mathrm{D}_{t,c}/\delta \mathrm{D}_{t,m} - 1)^2 + (\delta^{18}\mathrm{O}_{t,c}/\delta^{18}\mathrm{O}_{t,m} - 1)^2 \tag{4}$$

where the subscript $m$ is the measured values of the tree xylem water isotopic compositions and the subscript $c$ the calculated values by Equation (2) for given values of $f$. A variety of initial estimation of $f_1$ and $f_2$ were used to test the stability of the solution. The optimized values of $\delta \mathrm{D}_{\mathrm{t,c}}$ and $\delta^{18}\mathrm{O}_{\mathrm{t,c}}$ with the minimum $R$ indicate the water extraction patterns.

## RESULTS AND DISCUSSION

### *Seasonal variations of precipitation, soil moisture contents and predawn water potentials*

Figure 2 shows the precipitation, soil moisture contents and isotopic compositions of rain water. Rainfall was consecutive and intensive in July with an amount of 80·95 mm, offering more soil water for plant uptake. Meanwhile, all plant species had strong capacities of water uptake during the plant growing season in July. In September, monthly rainwater was reduced to 51·2 mm and the sampled soil was relatively dry after a non-raining period of 10 days. In December, rainfall was rare and its amount was only 20·20 mm. The extremely dry soil offered less water for plant uptake. During this time, plants were in the dormant period and took less water.

Figure 2(b) shows seasonal variations of soil moisture contents averaged at the sampling positions for the depths of 0–10 and 10–50 cm at the forest and bush sites. The soil moisture contents at the forest and bush sites exhibit similar temporal variations as rainfall does. Generally, the forest with its thick canopy decreases evaporative loss from the upper soil layer (Zimmermann *et al.*, 1966; Tiedemann and Klemmedson, 1977; Frost and McDougald, 1989; Thurow and Hester, 1997) by creating a microclimate of higher humidity and lower temperature beneath its leaves. In contrast, for the lower and sparsely distributed shrubs, the evaporative loss from the upper soil is greater. Thus, soil moisture content of the upper soil beneath forest was usually higher than that beneath the shrub (Figure 2). For the lower soil layer of 10–50 cm, it was wetter at the forest site than that at the bush site in the rainfall period of May to August (Figure 2). During the dry season, however, soil moisture content of the lower soil layer beneath forest was smaller than that beneath shrub, suggesting that evaporative loss from the lower layer by forest-cover exceeded the lower soil water loss beneath the shrub canopy (Figure 2).

PWPs of various species of plants in the study area were generally high because of humid subtropical monsoon climate zone where the study area was located (Table II). Generally, PWPs during the heavy rainfall period of July were higher than those in September and December. The lower PWPs in the rainfall season of September was attributed to that the soil at the sampling time was extremely dry after a non-raining period of 10 days (Figure 2). Generally, the decrease in PWPs from wet July to dry September and December was not as significant in other karst regions, e.g. pineland and

Table II. Mean and standard deviation (SD in parentheses) of PWPs for the plant species (MPa)

| Species | Seasons | Water potential (±SD) | Change in PWPs between wet and dry season(±SD) |
|---|---|---|---|
| *P. fortuneana* | July | −0·53(±0·04) | 0·09 (±0·07) |
| | September | −0·61(±0·06) | |
| | December | −0·62(±0·07) | |
| *C. pubescens* | July | −0·63(±0·08) | 0·12 (±0·09) |
| | September | −0·76(±0·05) | |
| | December | −0·75(±0·07) | |
| *P. longipes* | July | −0·49(±0·03) | 0·07 (±0·05) |
| | September | −0·56(±0·05) | |
| | December | −0·56(±0·09) | |
| *V. utile* | July | −0·44(±0·04) | 0·00 (±0·05) |
| | September | −0·46(±0·04) | |
| | December | −0·45(±0·07) | |
| *R. davurica* | July | −0·48(±0·06) | 0·05 (±0·06) |
| | September | −0·55(±0·05) | |
| | December | −0·53(±0·04) | |

hammock species in southern Florida (Ewe *et al.*, 1999), and ashe juniper and live oak in the eastern Edwards Plateau, TX, USA (Schwinning, 2008), indicating existence of stable water sources for plant uptake from the subcutaneous water at our study sites. The lowest PWPs and a largest decrease in PWPs from wet to dry season for *C. pubescens* of arbor, indicate that water sources for this species uptake is limited and unstable, which is accord with that its roots are not extended more deeply (Schwinning, 2008).

### *$\delta$D and $\delta^{18}$O values of soil water, subcutaneous flow and stem water*

In the subtropical monsoon region, the $\delta$D and $\delta^{18}$O values are significantly influenced by rainfall and present the seasonal variation (Figure 2). However, the local meteoric water line (LMWL) determined from local rainfall samples was indistinguishable from the global meteoric water line (Craig, 1961; Figure 3). Trend tendencies of the three water sources were similar with the LMWL, indicating that all sampled waters originally came from precipitation.

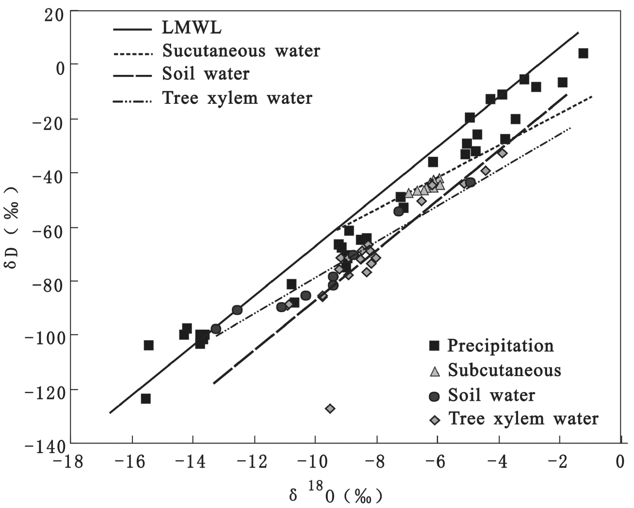

Figure 3. LMWL for 33 rainfall samples collected during November 2005 to December 2006. Also shown are the isotope ratios of water samples taken from other sources

The slopes of sampled water from the stem, the soil and the subcutaneous zone fell below the LMWL (Figure 3), indicating that all plants took up water predominantly from sources that were enriched by evaporation. As trend line of the $\delta$ value of the tree stem water was parallel to the LMWL and the $\delta$ value varied in a larger range as that of rainfall (Figure 3), the tree stem water shows a significantly seasonal variation along with rainfall. However, the variations of $\delta$ values of the water samples from the soils and the subcutaneous layer were affected by the isotope ratio between rainfall in the measured time and the original water stored in the layers (Ehleringer and Dawson, 1992). Fewer variations in isotope ratios of the three water sources are resulted from buffered effects of the soils and subcutaneous layer. The $\delta$ value of the subcutaneous water is more positive than that of the soil water because the perched water in the subcutaneous zone is more stagnant.

The $\delta^{18}$O and $\delta$D values from the soils and from the subcutaneous layer are very different as clearly shown in Figure 4. Given that D and $^{18}$O contents of the upper soil water were more enriched due to evaporation (Allison *et al.*, 1983), the upper layer of water became heavier than the lower layer of water (Sternberg *et al.*, 1991; Walker and Richardon, 1991; Smith *et al.*, 1997). The $\delta^{18}$O and $\delta$D values of the tree stem water fall mostly between those from the soil layer and the subcutaneous

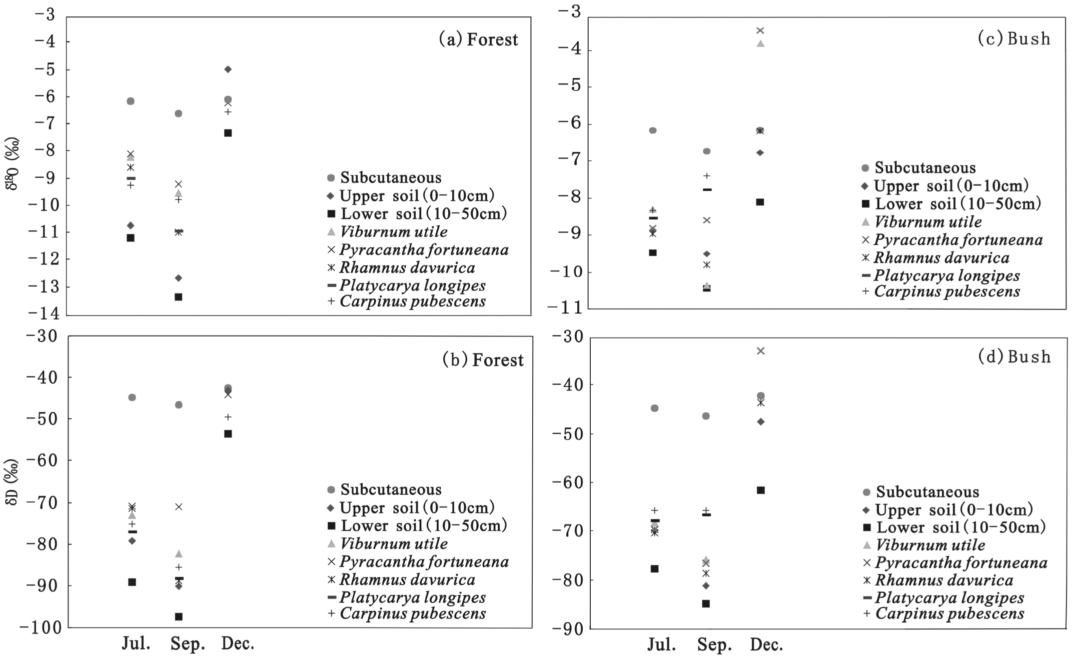

Figure 4. $\delta$D and $\delta^{18}$O values of twig xylem water, soil water and subcutaneous water at the forest and bush sites (a) $\delta^{18}$O at the forest site, (b) $\delta$D at the forest site, (c) $\delta^{18}$O at the bush site and (d) $\delta$D at the bush site

zone, a sign suggesting that the stem water came from the soil layer and the subcutaneous zone.

As shown in Figure 4, all the water sources had higher negative values for the $\delta$ measurements in the rainfall season of July and September than in the drought season of December. It was consistent with values reported for the influences in seasonal variations of precipitation and evapotranspiration in other studies (Liu *et al.*, 1997; Gat and Confiantini, 1981; Tu *et al.*, 2004). Compared to the seasonal variations of the $\delta$D and $\delta^{18}$O values from the soils water, less variation of these values from the subcutaneous water proved that the oxygen isotopic response at the spring was highly buffered (Perrin *et al.*, 2003).

Local environment conditions of land surface influence the $\delta$D and $\delta^{18}$O enrichment in the soils and the subcutaneous layer as well. Table I demonstrates that the $\delta$D and $\delta^{18}$O measurements for the soil water at the forest site had higher negative values than those at the bush site during the rainfall season of July and September but they were opposite during the drought season of December. The reason for this change is that during the rainfall season soils beneath the forest were relatively wetter and evaporative loss from the soils was smaller than that beneath the bush (Figure 2). In the drought season of December, however, soil became dry and most plant uptake was from the subcutaneous zone with the higher $\delta$D and $\delta^{18}$O values. Given that a relatively large evapotranspiration rate at the forest site, more evaporatively enriched subcutaneous water migrated upward and was supplied to the soil.

For different functional groups of the plant, Table I shows that the $\delta$D and $\delta^{18}$O measurements for the most stem xylem water at the forest site had higher negative values than those at the bush site. For the different species at the forest site, the $\delta$D and $\delta^{18}$O measurements for the tree group of arbor (*C. pubescens* and *P. longipes*) had higher negative values than those of the shrub (*V. utile, P. fortuneana* and *R. davurica*), indicating that the species of arbor consumed less subcutaneous water with more positive $\delta$D and $\delta^{18}$O values. At the bush site, however, it is opposite. The $\delta$D and $\delta^{18}$O values for the arbor were more positive than those of the shrub, indicating that the arbor consumed more water from the subcutaneous zone than from the shrub. Generally, for the sampled species, the influences in the plant growing environments on plant uptake are more important than that of the ecotype groups.

### *Contributions of the water sources for the plant uptake*

The contributions of different water sources for the sample species uptake were calculated by Equations (2)–(4) and are shown in Figure 5. The results demonstrate that water uptake for most plant species came from the upper and lower soils and the subcutaneous layer as well in this karst area. The proportions of the three water sources for plant uptake vary seasonally and depend on the selected plant species and the local environment conditions.

Proportions of water uptake from the upper and lower soil layers and the subcutaneous zone in July, September and December were shown in Figure 5(a) for the forest site and in Figure 5(b) for the bush site. For a presentation convenience, the individual plant species are grouped into arbor and shrub. In July, rainfall was the most intensive and plant uptake was strongest. For all species averaged at the forest site (Figure 5(a)), proportions of water uptake from the upper and lower soil layers and the subcutaneous zone were 27·73, 43·24 and 29·04%, respectively. In contrast, at the bush site, all plant species in average tended to take more water from the upper soil layer (Figure 5(b)). The proportions of water uptake from the upper and lower soil layers and the subcutaneous zone were 62·56, 28·40 and 9·03%, respectively.

In September, water uptake capacity of plant reduced along with the decrease in rainfall amount. Plant tended to take more water from the upper soil layer because a relatively weak water uptake capacity of plant existed but precipitation remained abundant during this period. Figure 5(a) shows that, for all species in average at the forest site, proportion of the water from the upper soil layer increased to 54·21%, and it reduced to 25·84 and 19·96% from the lower soil and subcutaneous layers, respectively; at the bush site (Figure 5(b)), 65·47% was from the upper soil layer, and 13·82 and 20·71% were from the lower soil and subcutaneous layers, respectively.

In the dry season of December, however, most water for plant uptake was from the subcutaneous layer. Generally, the plants consumed less water from the subcutaneous zone at the forest site than at the bush site. The subcutaneous water accounted for 67·84 and 98·66% of the total water sources for all species in average at the forest and bush sites, respectively (Figure 5(a) and (b)). In the drought season, larger trees are believed to have deeper rooting depths that take more water from the deeper soil (Schenk and Jackson, 2005). In the study region, however, larger trees of arbor at the forest site did not take any subcutaneous water, while the younger species at the bush site took more than 90% of subcutaneous water (see arbor in Figure 5(a) and (b)). This may be that roots in greater lateral spread or higher density in the thin soil layer could not reach the lower groundwater table because of larger evapotranspiration loss at the forest site for the dry climate. Our results strongly supported the hypothesis of the absence of a pronounced influence in plant size on water uptake depth in the karst region by Jackson *et al.* (1999a,b).

Figure 5(c) shows the proportions of the three water sources extracted by the species of shrub and arbor in terms of the site average. It demonstrates that the proportions of the subcutaneous water contribution for the shrub and arbor were not significantly different in July and September, but the shrub tended to take a larger proportion of the subcutaneous water in December. This may be attributed to different life-form of the sampled species. The selected species of arbor (*P. longipes and C. pubescens*) in the study area were deciduous hardwood, while two of the three species of shrub (*V. utile*

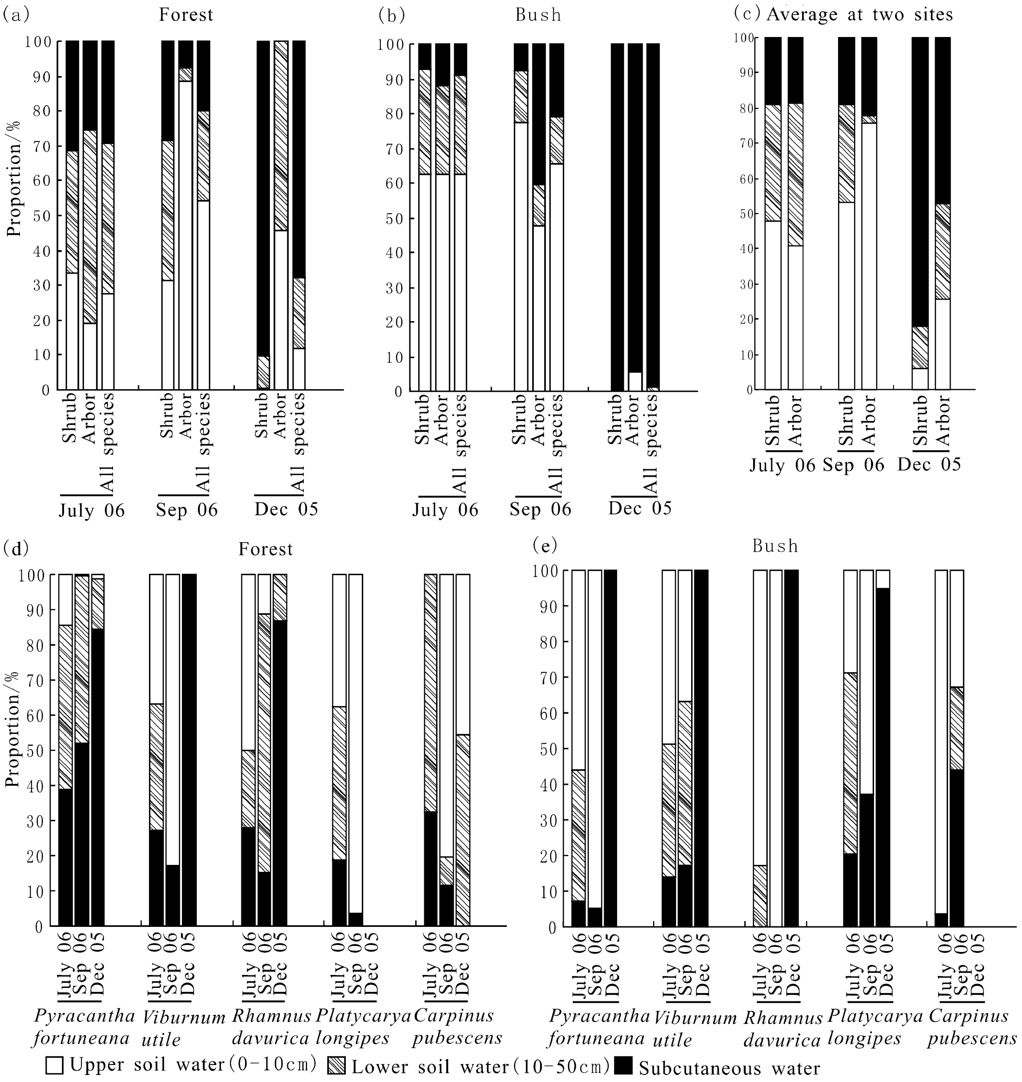

Figure 5. Proportions of water uptake from the upper and lower soil layers and the subcutaneous zone, (a) species in average for shrub and arbor at the forest site, (b) species in average for shrub and arbor at the bush site, (c) species averaged at the two sites for shrub and arbor, (d) different species at the forest site and (e) different species at the bush site

*and P. fortuneana*) were evergreen. As we know, in the drought season, deciduous species is dormant with defoliation and thus decreases water uptake, while evergreen species still need more stable water source, such as the subcutaneous water, for their growing (White *et al.*, 1985). This result supported the views of Jackson *et al.* (1995) and Meinzer *et al.* (1999) that evergreen tree species tap deeper sources of water, while drought deciduous ones obtain water from shallower soil layers, but it was contrary to the reports by Jackson *et al.* (1999a,b), Querejeta *et al.* (2007) and Stratton *et al.* (2000) that drought-deciduous tree species tended to rely on deeper water sources than evergreen species in a Brazilian Cerrado savanna, in a northern Yucatan and in a Hawaiian dry tropical forest, respectively. This controversy may result from different plant species and environmental conditions of soil and rocks. For example, investigated by Querejeta *et al.* (2007) in northern Yucatan, the evergreen *Ficus cotinifolia* and the drought-deciduous *Enterolobium cyclocarpum* were extracting water from depths >1 m, much deeper than our study region.

Detailed analysis for each species of plant at the forest and bush sites is shown in Figure 5(d) and (e), respectively. The subcutaneous zone contributed to water uptake for evergreen shrub of *P. fortuneana* and *V. utile* and for deciduous arbor of *P. longipes* at all sampling times, and this contribution was greater than 85% of the total water in December. In contrast, deciduous shrub of *R. davurica* in July and September at the bush site (Figure 5(e)) and deciduous arbor of *C. pubescens* in December at the forest site (Figure 5(d)) did not consume any subcutaneous water.

*P. fortuneana* at the forest site took a large proportion of the subcutaneous water during the three sampling periods of July, September and December, but at the bush site in July and September, this plant consumed a large proportion of water from the upper soil layer. For *V. utile*, 100% of water uptake was from the subcutaneous zone in December at the both sites. In July and September, however, more than 70% water came from the soil layers and the upper soil contributed more at the forest site than at the bush site. Uptake of the water sources for *P. longipes* at the forest and bush sites were similar to that for *V. utile*, except for that more water came from the upper soil layer and less from the subcutaneous zone at the forest site, while more water came from the subcutaneous zone at the bush site in July and September. For *R. davurica*, less water was taken from the subcutaneous zone in July and September. An extreme case at the bush site was that no water uptake came from the subcutaneous zone in July and September but all water came from the subcutaneous zone in December. *C. pubescens* was able to take the subcutaneous water in July and September at both sites, but it did not take any subcutaneous water in December at the forest site because of its shallower roots and weak capacity to take water from the deeper water table at the forest site in the drought season.

## SUMMARY AND CONCLUSIONS

In the karst region of southwest China, infiltrated water for plant consumption stores in the soil and rock fractures and tracing water sources for karst plants is very important to understanding water utilization strategies for plants in this fragile environmental region. In this study, twig xylem samples, together with the possible water sources, were collected at a forest and a bush site to determine the stable isotope compositions of water sources and to trace the sources from the soil zone and the subcutaneous zone.

Plant in the study region tends to first use the soil water rather than the underlying subcutaneous water. During the plant growing season of July, plants with strong uptake capacity consumed more water from the soil and less from the subcutaneous zone because more rainwater was available for storage in the soils. In the rainfall season of July and September, the younger plants of shrub at the bush site tended to take more water from the soils, especially from the upper soil, than those of the elders at the forest site. Even in the dry season of December, soil water provided more than 30% of plant uptake for the species of arbor and more than 15% for the species of shrub. In other tropical karst regions with shallow soils and seasonally dry climate (the Lower Florida Keys), Ish-Shalom *et al.* (1992) also found that hammock tree species primarily utilized soil water and not aquifer water. Therefore, prevention of soil erosion is very important for ecosystem recovery in the southwest karst region where about 41·2% of the land experiences soil erosion and loss of water because of deforestation and agriculture.

Water stored in the shallow fractured rock plays an important role in supporting tree transpiration during dry periods for ecosystems with shallow soils. In the dry season of December, shrubs took water from the subcutaneous zone accounting for 100% and more than 90% at the bush and forest sites, respectively.

Meanwhile, different plant species have different water uptake strategies in the drought climate. In the drought season of December, deciduous species is dormant with defoliation and thus decreases water uptake, while evergreen species still need more water for their growing. Especially, deciduous species of *C. pubescens* with shallower root and weak water uptake capacity is easily affected by climate variations. It is not able to tap the subcutaneous water in the dry season and plant PWPs are lower and decease from the wet to dry season more significantly than other species.

To our knowledge, this is the first isotopic study to address partitioning of water resources among native plant species in the southwest karst region. This study indicates that vertical partitioning of soil/bedrock water may decrease competition and promote plant species' coexistence in the region. The design and management of vegetation systems for environment recovery in this region should adapt to the environmental conditions, e.g. the spatial distribution characteristics of soil properties and rock fractures and their water storage contents in different seasons.

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