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## Water source utilization by woody plants growing on dolomite outcrops and nearby soils during dry seasons in karst region of Southwest China

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### SUMMARY

In the seasonally dry karst region of Southwest China, woodland vegetation is frequently associated with rocky outcrops. We used stable isotope techniques to determine the water sources of two woody plant species (the semideciduous tree Rademachera sinica and the deciduous shrub Alchornea trewioides) across three surface types: continuous and isolated dolomite outcrops, as well as adjacent surfaces with soils. Main water sources for R. sinica growing on the continuous outcrops shifted from deep water sources (e.g., water in the saturated zone) on March 23, 2009 (the late dry season of 2008-2009) to rainwater stored in rock fissures of the unsaturated zone on November 8, 2009 (the early dry season of 2009–2010). R. sinica growing on nearby thin soils exhibited a similar shift of main water sources, from deep water sources in the late dry season to shallow soil water (0-30 cm) in the early dry season. However, they extracted deep water sources from the bottom of nearby outcrops rather than from deeper layers below the soil surface. Main water sources for R. sinica growing on the isolated outcrops shifted from previous (one to two months ago) rainwater in the late dry season to the mixture of recent (within the last one month) and previous rainwater in the early dry season, while using little or no deep water sources. In contrast, A. trewioides growing on these two kinds of outcrops always relied on recent rainwater, while those growing on soils always relied on shallow soil water. The shift of water sources for tree species (especially those growing on continuous rock outcrops and nearby soils) may allow them to maintain normal transpiration throughout the year, which increases the amount of evapotranspiration in the watershed and further enhances the water storage capacity of the study area.

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### 1. Introduction

Southwest China is home to one of the largest karst regions (here and elsewhere) in the world, with an area of approximately 500,000 km<sup>2</sup> (Yuan, 1994). This area is characterized by rocky desertification with a high ratio of bedrock outcrop to shallow soil and low vegetation cover. The characteristics of rocky desertification together with the "dual" conduit and diffuse flow systems of karst aquifers contribute to the rapid hydrological processes (rapid penetration of rainwater through thin soil layer and connected fissures in bedrock layer to below the rooting zone or groundwater) of the area (Butscher and Huggenberger, 2009), as well as frequent flood/drought events.

Although this area is considered a subtropical region, with total annual precipitation exceeding 1000 mm, plants endure

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4–5 months of annual drought. Still, naturally reforested bedrock outcrops tend to thrive. Forest restoration increases the relative contribution of plant transpiration to evapotranspiration, and regulates the karstic hydrological process (Asbjornsen et al., 2011). Therefore, detailed observations of water sources used by rocky habitat plant species, especially during the dry season, can provide data for estimating effects of forest restoration on water balance. Many authors have revealed the type and diversity of plants growing on rock outcrops (Burgman, 1987; Anderson, 1999; Crow and Ware, 2007; Ware, 2010), however few have focused on sources of water used by plants and have examined the hydrological characteristics of these forests.

Plant water sources can be determined through stable isotope analysis of stem water (Dawson et al. 2002), and this technique has been widely used in determining water sources used by plants (Brunel et al., 1995; Meinzer et al., 1999; Schwinning et al., 2003; Querejeta et al., 2006, 2007; Asbjornsen et al., 2007; Hasselquist et al., 2010; Nie et al., 2011). There is no isotopic fractionation during water uptake by terrestrial plants, although examples of plant species that seem to fractionate hydrogen (but not oxygen) during

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water uptake exist (Lin and Sternberg, 1993; Ellsworth and Williams, 2007). Water sources can be pinpointed by comparing the isotope ratios of all potential water sources with water extracted from plant stems (Dawson et al., 2002).

Rose et al. (2003) found that both pine and manzanita growing on thin soil underlain by weathered granitic dolomite predominantly used surface soil water (0–75 cm), and exploited dolomite-derived water when soil water was depleted. McCole and Stern (2007) found that in thin soils over karstified carbonate bedrock, water sources of juniper changed from dominantly deep water during a hot, dry summer to dominantly soil water during a cool, moist winter. Schwinning (2008) found that tree species in karst savannas used water stored in the epikarst zone rather than groundwater. However, little is known about water sources utilized by plants growing on bedrock outcrops, or the difference between species growing on outcrops and nearby soils.

Rooting habit is an important factor affecting water use patterns of plants. Zwieniecki and Newton (1994, 1995) found that in thin soils underlain by thick zones of weathered bedrock, roots of some species were restricted in thin soil layer, while others could exploit water held in bedrock by growing roots into and following joint fractures. Zwieniecki and Newton (1996) verified that two conifer species showed limited ability to utilize water from bedrock even during dry periods. Ericaceous plants, on the other hand, were very efficient in taking water from deep rock layers, using nearly all available water and removing twice as much water as conifers of the same age. Hubbert et al. (2001) and Rose et al. (2003), both suggest that water stored in bedrock was especially important to pine relative to manzanita as active roots of manzanita were restricted to the upper 100 cm.

In karst regions, plant root distribution is strongly influenced by soil depth and epikarst structure (Schwinning, 2008). The epikarst (also known as the subcutaneous zone) is highly weathered carbonate bedrock that is either exposed or immediately beneath the surface (Williams, 2008). In some areas of Southwest China where bedrock is dominantly dolomite, soil is thin and the depth of the epikarst is usually restricted to a few meters, which hampers root growth (Cao and Yuan, 2005). However, when dolomite is exposed (usually manifested by ubiquitous cracks and crevices), it adds to the thickness of the epikarst. Thus, the same species growing in soils and on dolomite outcrops may have different water sources. Two kinds of outcrops are commonly encountered in dolomite-dominated areas of Southwest China, continuous and isolated. It is well known that water storage capacity of epikarst depends upon its thickness and continuity. Although there might be no difference of thickness between the epikarst of continuous and isolated outcrops, they could be separated from each other according to the continuity of their epikarst. Continuous outcrop could be regarded as a combination of dozens (if not hundreds) of isolated ones, water could transmit between adjacent ones through connected fissures under the influence of gravity and/or pore water pressure. The widely distributed lateral flow of water in continuous outcrop, contrary to the dominated vertical flow in isolated outcrop, extend the residence time of water in epikarst (which is equal to relatively high water storage capacity) and saturated zone is more likely to be formed in there. Thus, the same species on continuous and isolated outcrops may have different water sources. Roots of some species may penetrate through joint fractures and exploit water held there, while others may not. Species of different root systems in the same habitat may have different water sources, too. To test these theories, we conducted an isotopic study during Southwest China's dry seasons to determine the sources of water used by plants in different habitats. Two commonly encountered woody species, Radermachera sinica (a semideciduous tree that lose part of its foliage in the dry season) and Alchornea trewioides (a deciduous shrub but can leaf out at the

end of dry season if there is rain) were chosen. We hypothesized that the tree exhibits deeper root system than the shrub.

### 2. Materials and methods

### 2.1. Study area

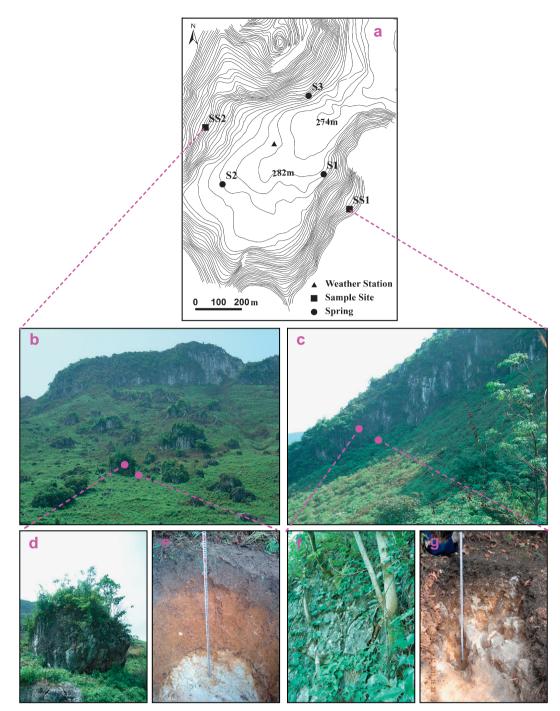
The study area is a small watershed (area =  $146.1 \text{ hm}^2$ ) located at the Huanjiang Observation and Research Station for Karst Ecosystems under the Chinese Academy of Sciences in Huanjiang County, Northwest Guangxi, China (24°43′58.9″–24°44′48.8″N, 108°18′56.9″-108°19′58.4″E). The watershed is a typical karstic peak-cluster depression area with a flat depression (area = 22.1 hm<sup>2</sup>) surrounded by mountain ranges on three sides and the mouth of the watershed in the northeast. Elevation ranges from 272.0 m to 647.2 m (Fig. 1a). A small stream flows from the southwest out of the watershed into a reservoir in the northeast. The soils are often saturated in a depression located in the southwest with a fluctuating groundwater table that is often below 1-3 m depth in the depression. Seepage springs sometimes appear at the bottom of hillslopes during the rainy season or after rains in the drought season. Hillslopes are characterized by steep slopes  $(62\% > 25^\circ)$ , shallow soils (10-30 cm deep except at the foot of)hillslopes and in the depression), and high dolomite outcrop ratio  $(\sim 30\%)$ . Shallow and discontinuous soils have been developed from dolomite and contain significant amounts of rock fragments. Soils are well-drained, gravelly and calcareous, and have a clay to clay loam texture (25-50% silt and 30-60% clay). The steadystate infiltration rate measured with a disc permeameter is  $0.7-2.1 \text{ mm min}^{-1}$  on the hillslopes (Chen and Wang, 2008). Organic matter content is relatively high ranging from 2.2% to 10.1% while PH varies between 7.1 and 8.0 (Chen et al., 2011). A subtropical mountainous monsoon climate dominates with annual rainfall of 1389.1 mm and annual air temperature of 18.5 °C. The wet season occurs between the end of April and early September. Plants usually experiences a pronounced 4–6 month dry season in winter/spring during which only 20-30% of the total annual rainfall is received.

This area experienced severe deforestation from 1958 to the mid-1980s, and has been under natural restoration for almost 25 years. Currently, the vegetation can be classified into three secondary communities: tussock, shrub, and secondary forest. Almost 70% of hillslopes are dominated by tussocks and shrubs. Most trees are found on dolomite outcrops and nearby soils, or in the deep soils (>100 cm) at the foot of hillslopes. Dolomite outcrops in this area can be divided into two types: continuous and isolated (Chen et al., 2011). Both continuous and large isolated outcrops are severely weathered and characterized by a network of soil-filled (may be litter or humus) cracks, crevices, and channels, with no soil in any place other than the network (Nie et al., 2011). Plant species growing on outcrops usually emerge from cracks or crevices (Fig. 1f), or grow on protuberant rocks with their roots ultimately penetrating into cracks.

### 2.2. Sampling sites characteristics

According to the distribution of these two types of outcrops, one continuous outcrop (Sampling site 1, SS1) was chosen at an upper slope position of a Northwest-facing hillslope, and one isolated outcrop (Sampling site 2, SS2) was chosen at a lower slope position of the opposite hillslope (Fig. 1a). There are continuous outcrops present at both the upper and lower positions of the Northwest-facing hillslope. The upper outcrop starts at the top of the hill and is about 80 m in length along the slope (Fig. 1c). The average height from the top of the outcrop to nearby soil is 7 m, and

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**Fig. 1.** Distribution of sampling sites and pictures of different habitats: (a) Distribution of sampling sites within the watershed; (b) Distribution of isolated bedrock outcrops on the Southeast-facing hillslope; (c) Distribution of continuous bedrock outcrops along the Northwest-facing hillslope; (d) Plants growing on the isolated bedrock outcrop; (e) Soil profile near the foot of the isolated bedrock outcrop; (f) Plants growing on the continuous bedrock outcrops; and (g) Soil profile near the foot of the continuous bedrock outcrops.

average width of the outcrop is 20 m. The outcrop is covered by dense vegetation (Fig. 1f), with *R. sinica* and *Celtis biondii Pamp* dominating the tree layer. *A. trewioides* and *Loropetalum chinense* dominate the shrub layer. Soil at the foot of the outcrop is shallow (about 30 cm deep) and discontinuous, horizontally interrupted by small outcrops, and vertically interrupted by big rocks (Fig. 1g). These two distinct habitats both contain *R. sinica* and *A. trewioides*. The opposite hillslope is covered by tussocks and shrubs, with many isolated dolomite outcrops of various sizes along the slope (Fig. 1b). A cube-shaped outcrop at the bottom of the slope with a rough surface of about 100 m<sup>2</sup> (about 8 m in length and 12.5 m

in width) and approximately 8 m high was chosen for the study (Fig. 1d). The tree layer is dominated by *R. sinica* and *Sterculia nobilis*, and the shrub layer is dominated by *A. trewioides* and *Vitex. Negundo Linn.* Soils around the outcrop are deep (80–100 cm) and clayey with a layer of highly weathered bedrock about 30 cm thick and crumbly by bare hand below the soil (Fig. 1e). This severely weathered bedrock is followed by a weakly weathered layer. The absence of trees in this habitat may result from human disturbance.

In addition, three spring outflow points were selected. Spring 1 (S1) is located at the bottom of the Northwest-facing hillslope,

Spring 2 (S2) is located in the depression near the bottom of the other selected hillslope (Southeast-facing), Spring 3 (S3) is at the lower slope position of a Southeast-facing hillslope about 1 km away from S2 (Fig. 1a).

### 2.3. Water sources in the environment

In karst regions, plants growing on outcrops and surrounding soils are likely to have deep roots and extract groundwater or water from the saturated zone, but, in this case, spring water is the only easily accessible source of deep water (Bonacci, 2001; White, 2002; Bakalowicz, 2005). We assumed that isotopic compositions of deep water sources were relatively stable and could be distinguished from other sources (e.g., rainwater and soil water) during most of the year. Thus, isotopic composition of spring water could be used to represent that of deep water sources potentially accessed by plants. Rainwater was treated as a potential water source for plants growing on outcrops as they can use rainwater stored in crevices/cracks directly. Dolomite outcrops in the study area were severely weathered and manifested by ubiquitous cracks and crevices, with most rainwater flowing along connected fissures into deeper layers without time delay and mixing with other kinds of water. However, a small part of rainwater may be stored in tightly closed fissures. Rainwater stored in cracks and crevices had not undergone evaporative enrichment, mixing with other kinds of water sources (Stothoff et al., 1999), or been influenced by too much evaporation (Querejeta et al., 2006). Since most plants absorb water from shallow layers when it is available (Schenk, 2005), water stored in upper layer rock fissures should be depleted more rapidly than that in deeper layers and dominated by recent rainwater. Thus recent (within the last one month) rainwater was used to represent water stored in rock fissures of the upper layers, while previous (one to two months ago) rainwater was used to represent water in deeper layers of the unsaturated zone. Both spring water and soil water at different depths throughout the soil profile were treated as potential water sources for species growing on soils.

### 2.4. Precipitation and spring water sampling

Monthly rainfall samples were routinely collected from November 2008–November 2009, except December 2008, Jan. 2009, and February 2009. The collection equipment was designed according to the new device for monthly rainfall sampling for the Global Network of Isotopes in Precipitation (IAEA, 2002). Samples for main rain events between November 1, 2008 and March 23, 2009 (the first plant sampling date) and between October 1, 2009 and November 8, 2009 (the second plant sampling date) were collected separately. Rainwater for single rain events was collected in a plastic tank with safeguards to prevent evaporation (Li et al., 2007). Spring water was sampled regularly both in the two dry seasons and the wet season between November 2008 and November 2009. Both rainwater and spring water were stored in cap vials, wrapped in parafilm, and frozen until stable isotope analysis.

### 2.5. Plant and soil sampling

Plant sampling was conducted separately on March 23, 2009 (late dry season of 2008–2009) and November 8, 2009 (early dry season of 2009–2010). At SS1, the two most frequently encountered species (*R. sinica* and *A. trewioides*) both growing on the continuous outcrop (SS1-outcrop) and in nearby thin soils (SS1-soil) were sampled. At SS2, *A. trewioides* growing on the isolated outcrop (SS2-outcrop) and in nearby soils (SS2-soil) was sampled, while *R. sinica* was sampled on the outcrop. Suberized twigs were cut from the canopy (using high sticks scissors) and leaves and green stem

tissue were removed to avoid contamination of stem water by isotopically enriched water (Ehleringer and Dawson, 1992; Querejeta et al., 2006). Three adult plants were selected per species per habitat. Clipped twigs were immediately placed in a capped vial and wrapped in parafilm. In the laboratory, samples were frozen and stored.

Soil samples were collected with a soil auger. At each sampling time, three locations were randomly chosen per sampling site. Soil samples were collected from depths of 0–5, 5–10, 10–20 and 20–30 cm at SS1, and from depths of 0–5, 5–10, 10–20, 20–30, 30–50, 50–70, 70–90 and 90–100 cm (the severely weathered dolomite layer) at SS2. All soil samples were placed in capped vials, wrapped in parafilm, frozen, and stored.

### 2.6. Root investigation

According to their distribution, eight *R. sinica* and fifteen *A. trewioides* plants growing on soils were selected for root investigation. Entire root systems were excavated by manual digging and their characteristics were recorded (e.g., root style, maximum vertical depth, and maximum root length). Characteristics of *R. sinica* roots growing on outcrops were recorded by visual inspection made in roadcuts near the study area. Root characteristics of *A. trewioides* growing on outcrops were observed in whole root systems extracted from cracks. The characters of coarse and fine roots were recorded and lost roots were estimated from the size and number of break-points.

### 2.7. Lab analysis

Stem and soil water were extracted by Ehleringer et al.'s (2000) cryogenic vacuum method. Samples were measured with a Finnigan MAT Delta V advantage isotope ratio mass spectrometer at the Stable Isotope Ratio Mass Spectrometer Facility, Chinese Academy of Forestry. Isotopic composition of oxygen is expressed in delta notation ( $\delta$ ) as

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \tag{1}$$

where  $\delta$  is the differential isotope value of the sample relative to standard (SMOW), and *R* is the absolute isotopic ratio (<sup>18</sup>O/<sup>16</sup>O) of the sample or standard. Analytical precision was better than 0.2‰.

### 2.8. Data analysis

The most probable sources of water up-take, was found by using oxygen isotopic composition to compare stem water with potential water sources of spring water, rainwater (plants growing on outcrops), or soil water (plants growing on soils) in order to identify the best 'match'. This approach was named by Asbjornsen et al. (2007) as "Direct Inference". In this approach, stem water of each plant was treated separately and the average  $\delta^{18}$ O values of spring water (on plant sampling day) of the three outflow points were used. Rainwater of the sampled event rainfall before the plant sampling day was treated as a single water source, while monthly rainwater received just before rain events was treated as a mixture. According to soil sampling increments, soil water of each layer was treated as a single water source.

As contributions of water from different water sources could account for the same stem water isotope values, actual plant water sources may be misinterpreted based only on the direct inference approach (Asbjornsen et al., 2007). Therefore,  $\delta^{18}$ O values of potential water sources and stem water were analyzed with the Iso-Source model (Phillips and Gregg, 2003) to evaluate relative contribution of each source to stem water. Spring water and rainwater were treated in the same way as the former approach. Average isotope values/species/habitat were used in the model, instead

of treating each plant individually. The number of potential water sources was reduced and the results were simplified by combining adjacent soil layers into a single layer if they had the similar isotope values (Phillips et al., 2005). To guarantee that no legitimate feasible source combinations was missed, the fractional increment employed in our calculations was 0.2, and uncertainty or "mass balance tolerance" was not smaller than half this amount (0.5 × increment × maximum difference between sources) (Phillips and Gregg, 2003). Uncertainty level was set at 0.2‰ in our calculations.

### 3. Results

# 3.1. Precipitation distribution and isotopic compositions of rainwater and spring water

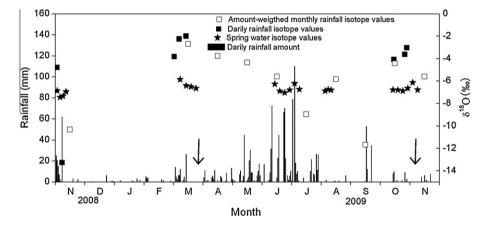
Fig. 2 shows rainfall measured in the study area and  $\delta^{18}$ O values of rainwater (both amount-weighted monthly rainfall and event rainfall) and spring water from November 2008–November 2009. The study area received 139 mm of rainfall in November 2008, 2.6 times the long-term mean monthly rainfall amount. 26% of long-term mean precipitation was received in a wet month followed by severe drought (December 2008–February 2009). The continuous drought was terminated by rain events (78.8 mm total) in early March 2009. The first plants/soil sampling was conducted in the ten clear days following the last rainfall (received March 13, 2009).

The study area received 1101 mm of rainfall during the wet season (between April and September) of 2009, which was 81 mm less than the long-term mean rainfall amount, with three main rain events in October 2009 (24.4 mm in total). The second plant/soil sampling was taken place in during the twelve clear days after the last rainfall (received on October 27, 2009).

On the one hand, the amount-weighted monthly rainfall mean  $\delta^{18}$ O values varied widely throughout the 10 months, with more negative values during the wet season and more positive values during the dry seasons (reflecting the well-known seasonal effect). However, rainwater in November 2008 exhibited the second lowest  $\delta^{18}$ O value among the 10 months, reflecting the so-called amount effect. Event rainfall sampled within month showed relatively small variation, except the two main rain events sampled in November 2008. On the other hand, spring water  $\delta^{18}$ O values exhibited very small variation with no obvious differences throughout the sampling time.

### 3.2. Water source determination

Direct inference and the IsoSource model were used to determine plant water uptake. In these two approaches, spring water and rainwater, during different time periods, were considered potential water sources for species growing on outcrops, while spring water and soil water, at different depths, were considered potential water sources for species growing on soils. On March 23, 2009, previous rainwater was excluded from the potential water sources for



**Fig. 2.** Distribution of daily rainfall and fluctuation of  $\delta^{18}$ O values of amount-weighted monthly rainfall from November 2008–November 2009, as well as  $\delta^{18}$ O values of event rainfall and spring water. Arrows indicate sampling times. *Error bars* represent SE.

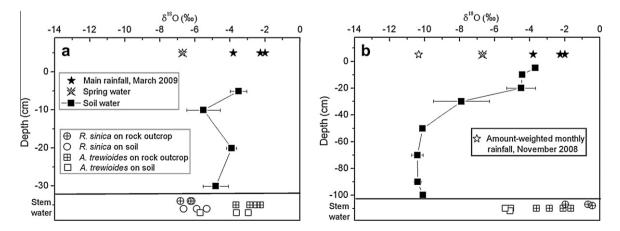


Fig. 3. Isotopic values for stem water and potential water sources for plants growing on dolomite outcrops on March 23, 2009 (a, plants growing on the continuous outcrops; b, plants growing on the isolated outcrops). Isotopic values for individual *R. sinica* and *A. trewioides* are shown beneath those for the soil profile. *Error bars* represent SE.

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#### Table 1

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Proportions of feasible water sources (%)<sup>a</sup> *R. sinica* (tree) and *A. trewioides* (shrub) growing on outcrops and nearby soils at two sampling site (SS1 and SS2) on March 23, 2009 (late dry season of 2008–2009). Oxygen isotope values were used for the calculations. Average source proportions calculated by the model are shown, as well as range of minimum/ maximum source proportions (*in parentheses*).

	SS1-rock Tree	SS1-soil Tree	SS1-rock Shrub	SS1-soil Shrub	SS2-rock Shrub	SS2-soil Shrub
Rain1(March 2) <sup>b</sup>	4		19	-	14	_
(-3.8‰) <sup>c</sup>	(0-16)		(0-54)		(0-44)	
Rain2(March 6)	3		37	-	39	-
(-2.3%)	(0-10)		(0-92)		(0-96)	
Rain3(March 13)	2		36		41	
(-2.0%)	(0-10)		(0-86)		(0-90)	
Spring	91	61	8	5	6	15
(-6.7%)	(84-98)	(26-84)	(0-20)	(0-24)	(0-16)	(0-56)
SS1-soil (0–5 cm) <sup>d</sup>	-	6	-	40	-	-
(-3.5%)		(0-28)		(0-86)		
SS1-soil (5-10 cm)	-	16	-	9	-	-
(-5.5%)		(0-74)		(0-40)		
SS1-soil (10-20 cm)	-	7	-	32	-	-
(-3.9%)		(0-32)		(0-100)		
SS1-soil (20–30 cm)	-	10	-	14	-	-
(-4.8%)		(0-46)		(0-60)		
SS2-soil (0–5 cm)	-	-	-	-	-	36
(-3.7%)						(0-80)
SS2-soil (5-20 cm)	-	-	-	-	-	33
(-4.4%)						(0-88)
SS2-soil (20-30 cm)	-	-	-	-	-	10
(-7.9%)						(0-40)
SS2-soil (30-100 cm)	-	-	-	-	-	6
(-10.3‰)						(0-24)

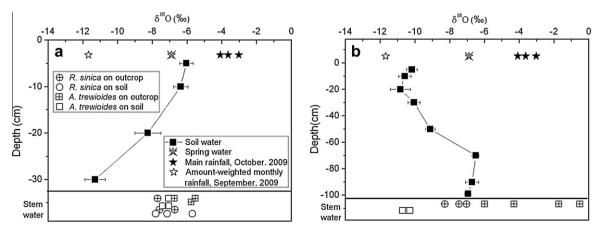
<sup>a</sup> Water source proportions were calculated using the IsoSource model (Phillips and Gregg, 2003), Rainwater and spring water were considered as the potential sources for plants growing on outcrop, while spring water and soil water at different depths were the potential sources for plants growing on thin soils.

<sup>b</sup> Date in parenthesis indicate that rainwater was received on that day.

 $^{c}\,$  Negative values in parenthesis indicate the  $\delta^{18}O$  value of water specified in the table.

<sup>d</sup> Soil water isotopic values represent average values of depth intervals specified in the table.

plants growing on rock outcrops as there was a long drought period before March 2009 and water stored in rock fissures of the unsaturated zone should have been recharged from recent rainwater. On November 8, 2009, previous rainwater was considered as a potential water source for plants growing on rock outcrops, as isotope values of stem water exceeded the range between spring water and recent rainwater. Considering the relatively low water storage capacity of rock fissures, the amount-weighted mean isotopic composition of rainwater received in September 2009 was used to represent that of previous rainwater. As described above, actual plant water sources may be misinterpreted based on the direct inference approach. Thus, when there was a conflict between the results of these two approaches, our conclusion was subject to the result of IsoSource model. Fig. 3a shows the distribution of  $\delta^{18}$ O values for plants sampled at SS1 on March 23, 2009 and their corresponding potential water sources. The distributions of oxygen isotope values for *R. sinica* (tree) on SS1-rock match those of spring water, indicating that they rely on deep water sources. IsoSource outputs also showed that these plants used very large proportions of spring water (average 91%, Table 1). Soil water throughout the soil profile at SS1 was more depleted of heavy isotopes than recent rainwater and had no regular relationship with depth. One *R. sinica* on SS1-soil had a similar  $\delta^{18}$ O value to spring water, while values for the other two were more positive than those of spring water and seemingly matched those of soil water at a depth of 5–10 cm. IsoSource outputs showed that water sources for these plants were still dominated by spring water (average 61%, Table 1). Oxygen isotope



**Fig. 4.** Isotopic values for stem water and potential water sources for plants growing on dolomite outcrops on November 8, 2009 (a, plants growing on the continuous outcrops; b, plants growing on the isolated outcrops). Isotopic values for individual *R. sinica* and *A. trewioides* are shown beneath those for the soil profile. *Error bars* represent SE

values for *A. trewioides* (shrub) on SS1-rock and SS1-soil had a greater spread than those of *R. sinica.* However,  $\delta^{18}$ O values for individuals in the former habitat plotted within the range of recent rains and individuals in the latter habitat plotted within those of soil water at a 0–30 cm depth. Furthermore, IsoSource outputs also showed that *A. trewioides* in the above two habitats obtained large proportions of recent rainwater (average 92% total) and soil water (average 95%), respectively (Table 1).

Fig. 3b shows the distribution of  $\delta^{18}$ O values for plants sampled at SS2 on March 23, 2009 and corresponding potential water sources. Oxygen isotope value for one R. sinica on SS2-rock matched those of recent rainwater, while the other two exhibited very positive isotope values, beyond the confine of those of potential water sources. Thus, there was no IsoSource solution for R. sinica on SS2-rock. Oxygen isotope values for A. trewioides on SS2-rock plotted within the range of recent rains. IsoSource outputs also showed that these plants used a large proportion of recent rainwater (average 94% total, Table 1). At SS2,  $\delta^{18}$ O values of soil water within the 0-20 cm depth were similar to those of recent rains, indicating that soil water had been recharged (or replaced) by these rains. A shaper decline of soil water  $\delta^{18}$ O values from 20 to 50 cm may reflect the evaporative enrichment of heavier isotopes, the input of isotopically heavy rains (recent rains), or the mixing of soil water stored above the 20 cm depth (through a piston flow mechanism). Soil water  $\delta^{18}$ O values were very uniform below a depth of 50 cm and similar to previous rainwater (rainfall received in November 2008). Oxygen isotope values for A. trewioides on SS2soil clustered and matched those of soil water above a 30 cm depth. IsoSource outputs also showed that these plants derived most of the water they used from this layer (average 85% total, Table 1).

Fig. 4a shows the distribution of  $\delta^{18}$ O values for plants sampled at SS1 on November 8, 2009 and corresponding potential water sources. Oxygen isotope values for *R. sinica* on SS1-rock matched that of spring water and plotted between those of recent rainwater and previous rainwater simultaneously. In this case, it is not easy to draw definite conclusions about source of water uptake by plants based on direct inference. IsoSource outputs showed that they used a large proportion of recent rainwater (average 40%) and previous rainwater (average 36%) at the same time (Table 2). Soil water  $\delta^{18}$ O values declined sharply throughout the thin soil layer, but were always more negative than recent rainwater, indicating that soil water was still dominated by previous rainwater. Oxygen isotope values for *R. sinica* on SS1-soil had a larger spread than those on SS1-rock. Direct inference was invalid because  $\delta^{18}$ O values for these plants plotted in the range of soil water or close to spring water. IsoSource outputs showed that these plants used the largest proportion of soil water (average 64% total, Table 2). A. trewioides on SS1-rock exhibited more negative  $\delta^{18}$ O values than recent rainwater: however, IsoSource outputs showed that they still used a large proportion of recent rainwater (average 55% total. Table 2). A. trewioides on SS1-soil had similar  $\delta^{18}$ O values to R. sinica in the same habitat, and IsoSource outputs showed that they also used a large proportion of soil water (average 62% total, Table 2).

Fig. 4b shows the distribution of  $\delta^{18}$ O values for plants sampled at SS2 on November 8, 2009 and corresponding potential water sources. Oxygen isotope values for *R. sinica* on SS2-rock were more negative than those of recent rainwater and plotted between those of spring water and previous rainwater. IsoSource outputs showed that they used a large proportion of previous rainwater (average 40%) and a comparative proportion of recent rainwater (average 39% total) at the same time (Table 2). Oxygen isotope values for *A. trewioides* on SS2-rock had a wide spread, with two being more negative and the two being more positive than recent rainwater. IsoSource outputs showed that they used the largest proportion of recent rainwater (average 91% total, Table 2) based on average  $\delta^{18}$ O values of their stem water. Soil water  $\delta^{18}$ O values gradually declined in the upper 20 cm of the profile but were much closer to previous rainwater rather than recent rainwater. Oxygen isotope

### Table 2

Proportions of feasible water sources (%)<sup>a</sup> *R. sinica* (tree) and *A. trewioides* (shrub) growing on outcrops and nearby soils at two sampling site (SS1 and SS2) on November 8, 2009 (early dry season of 2009–2010). Oxygen isotope values were used for the calculations. Average source proportions calculated by the model are shown, as well as the range of minimum/maximum source proportions (*in parentheses*).

	SS1-rock Tree	SS1-soil Tree	SS1-rock Shrub	SS1-soil Shrub	SS2-rock Tree	SS2-rock Shrub	SS2-soil Shrub
Rain1 (September) <sup>b</sup>	36	-	19	-	40	3	-
(-11.7‰) <sup>c</sup>	(6-50)		(0-36)		(12-54)	(0-12)	
Rain2 (November 14)	15	-	20	-	14	24	-
(-4.1%)	(0-60)		(0-76)		(0-56)	(0-100)	
Rain3 (November 25)	13	-	18	-	13	31	-
(-3.6‰)	(0-56)		(0-72)		(0-52)	(0-98)	
Rain4 (November 27)	12	-	17	-	12	36	-
(-3.0%)	(0-52)		(0-66)		(0-48)	(0-90)	
Spring	24	36	26	38	21	6	3
(-6.9%)	(0-94)	(0-90)	(0-82)	(0-96)	(0-88)	(0-28)	(0-10)
SS1-soil (0–10 cm) <sup>d</sup>	-	34	-	41	-	-	-
(-6.2%)		(0-78)		(0-84)			
SS1-soil (10-20 cm)	-	20	-	14	-	-	-
-9.0%)		(0-54)		(0-44)			
SS1-soil (20–30 cm)	-	10	-	7	-	-	-
(-11.2%)		(0-28)		(0-22)			
SS2-soil (0–30 cm)	-	-	-	-	-	-	87
(-10.4‰)							(70-100)
SS2-soil (30–50 cm)	-	-	-	-	-	-	7
(-9.1‰)							(0-30)
SS2-soil(50–100 cm)	-	-	-	-	-	-	3
(-6.7‰)							(0-10)

<sup>a</sup> Water source proportions were calculated using the IsoSource model (Phillips and Gregg, 2003), Rainwater and spring water were considered as the potential sources for plants growing on the outcrop, while spring water and soil water at different depths were the potential sources for plants growing on thin soils.

<sup>b</sup> Date or month in parenthesis indicate that rainwater was received on that day or in that month.

<sup>c</sup> Negative values in parenthesis indicate the  $\delta^{18}$ O value of water specified in the table.

<sup>d</sup> Soil water isotopic values represent average values of depth intervals specified in the table.

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values increased from -10.83% at 20 cm depth to -6.51% at 70 cm depth and exhibited similar isotope values to spring water below 70 cm depth. Oxygen isotope values for *A. trewioides* on SS2-soil clustered and matched those of soil water above 30 cm depth. IsoSource outputs also showed that these plants derived almost all the water they used from this layer (average 87%, Table 2).

### 3.3. Root distribution

A. trewioides growing in soils exhibited a typical shallow root system. Although height of excavated individuals varied from 130 cm to 300 cm, most of their roots were restricted within the upper 30 cm. Main roots were horizontally oriented, with the longest observed root being 140 cm away from the base of the trunk. In addition, all coarse roots were surrounded by a high density of fine roots (<1 or 2 mm in diameter). Roots of *R. sinica* growing on soils (deep soil habitat was excluded from the investigation) were also concentrated in upper soil layers, with only a few relatively small roots (<30 mm in diameter) penetrating vertically into lower layers (Fig. 5). Although these horizontally oriented roots spread far from the base of trunks (some spread further than 5 m), most of them were limited within 1 m vertically. In addition, most of these roots finally penetrated into the bottoms of nearby dolomite outcrops. Contrary to the high density of fine roots for A. trewioides, *R. sinica* had a relatively low density of fine roots in the soil layers.

*A. trewioides* growing on outcrops also exhibited shallow root systems. The whole coarse root system (more than 2 mm in diameter) of a large proportion of plants could be pulled out manually without tools. Root broken-points were occasionally encountered because of this rude investigation method; however, most of them were in small sizes and had no effect on judging the style of the root system. In addition, *A. trewioides* growing on outcrops had a high density of fine roots. Roots of tree species, such as *R. sinica*, growing on outcrops could penetrate through soil-filled cracks and crevices into deep layers (Stothoff et al., 1999); however, we had no robust information to support this result.

### 4. Discussion

### 4.1. Potential water sources

### 4.1.1. Rainwater and spring water

The amount-weighted monthly rainfall mean  $\delta^{18}\text{O}$  values varied widely from November 2008-November 2009 and reflected a wellknown seasonal effect, while event rainfall sampled within month showed relatively small variation (Fig. 2). Similar results have also been obtained in many other regions around the world (Ingraham et al., 1991; Jones and Ingraham et al., 2004; Yamanaka et al., 2007). Generally, plants take up water derived from rainwater from different kinds of water-bearing media, however, species growing on rock outcrops may use rainwater stored in bottomclosed cracks/fissures or intercept slow moving rainwater within fissures. Thus, rainwater of different time periods were considered as potential water sources for plants growing on rock outcrops in this study. In fact, many previous studies took rainwater as a potential water source for plants in different habitats (Phillips and Ehleringer, 1995; Williams and Ehleringer, 2000; Schwinning, 2008).

Oxygen isotope values of spring water exhibited relatively steady  $\delta^{18}$ O values throughout the sample period (Fig. 2). The small variation of  $\delta^{18}$ O values of spring water have been discussed in our previous paper (Nie et al., 2011). Isotopic compositions of spring water, first used by McCole and Stern (2007) in determining water sources of plant in karst region, was used to represent those of deep water sources that are potentially accessed by plant.

### 4.1.2. Soil water

On March 23, 2009, oxygen isotope values for soil water sampled at SS1 plotted between those of recent rainwater and spring water and had no regular relationship with depth (Fig. 3a). Theoretically,  $\delta^{18}$ O values for soil water should be more positive than those of recent rainwater, or at least within the range of those of recent rainwater (Nie et al., 2011). The possibility of hydraulic lift



Fig. 5. Horizontal distribution of main roots for *R. sinica* growing on thin soils (a, main roots for *R. sinica* growing on thin soil underlain by weakly weathered bedrock; b, main roots for *R. sinica* growing on thin soils underlain by consolidated bedrock; c, main roots for *R. sinica* growing on loose rocky soil).

was excluded and one hypothesis, spring water recharged soil water, was developed to explain the unusual distribution of soil water  $\delta^{18}$ O values. In fact, we observed seepage of water from fissures (located at the lower part of the outcrop) after rainstorms at SS1, and the isotope values were similar to the spring water (data not shown). Thus, we believe that there was also seepage of water from fissures (located at different depths below soil surface) that infiltrated into nearby soils. Consequently, there are horizontal gradients of water potential between soils at different distances from the seepage point, and seepage water may horizontally diffuse to nearby soils and change their isotope values (Liu et al., 1994; Castiglione et al., 2003). The irregular distribution of soil water  $\delta^{18}$ O values with depth may result from soil samples that were collected at different distances from the outcrop and seepage points being distributed randomly at different depths. Conversely, oxygen isotope values for soil water sampled on November 8, 2009 at SS1 declined regularly with depth (Fig. 4a), however: this result could not exclude the possibility that there was seepage of water from fissures at the sampling time. There may be lack of water potential gradient, as the study area was much wetter before the second sampling time than before the first sampling time (Fig. 2).

On March 23, 2009,  $\delta^{18}$ O values of soil water at SS2 formed a typical isotope profile (soil water isotope values gradually declined with the depth, Fig. 3b). A similar isotope profile was observed both in karst regions (Querejeta et al., 2007; Hasselquist et al., 2010) and non-karst regions (Meinzer et al., 1999; Liu et al., 2010). The less negative  $\delta^{18}$ O values in upper soil layers can be explained by evaporative enrichment of heavier isotopes or the input of isotopically heavier rainfall. However, the extremely negative and uniform  $\delta^{18}\text{O}$  values of the deeper layers could only be related to rainfall received at least three months ago (November 2008), which is consistent with results obtained in other studies (Schulze et al., 1996; Gazis and Feng, 2004; Yang et al., 2011). Oxygen isotope values of soil water sampled on November 8, 2009 at SS2 formed a much more complicated isotope profile (Fig. 4b). Soil water  $\delta^{18}$ O values declined with depth in the upper 20 cm, increased gradually with depth between 20 and 70 cm, and varied little between 70 and 100 cm. It is difficult to distinguish water sources for each soil layer; further research should focus on redistribution of water in deep soil habitats of karst region.

### 4.2. Plant water use patterns

R. sinica on SS1-rock relied on deep water sources (represented by spring water) in the late dry season (March 23, 2009) and relied on rainwater stored in rock fissures of the unsaturated zone in the early dry season (November 8, 2009). This difference has also been observed between the dry and wet season (Nie et al., 2011). These variations of main water sources indicate that R. sinica on SS1-rock has a dimorphic root system, with shallow roots using rainwater mainly stored in bottom-closed cracks or slowly moving water within fissures, while deep roots extracted water from deeper layers. Generally, plant roots extract resources from shallow layers (when it available) to minimize plant energy expenditure (Schenk, 2005). The study area received a large amount of rainwater in the two months before November 2009, therefore, enough rainwater should be stored in bottom-closed cracks to support R. sinica's transpiration demand. Many studies found that species growing on thin soils underlain by deep weathered bedrock layers primarily extract soil water when available (Rose et al., 2003; McCole and Stern, 2007). There were also several rain pulses in the early part of March 2009, however, R. sinica on SS1-rock made no use of these rains. There are two possible reasons for this result. Firstly, studies have found that deep-rooted species may extract precipitation from upper layers when amounts were above some minimum threshold (Williams and Ehleringer, 2000; West et al., 2007) (i.e.,

rainfall amounts in early March 2009 was not enough). Secondly, fine roots in upper layers may have senesced and shed during long dry time periods (Dawson and Pate, 1996) with new sets of fine roots not being developed before sampling.

Main water sources for R. sinica on SS1-soil exhibited a similar pattern to those on SS1-rock, from deep-water sources in the late dry season to shallow soil water in the early dry season. The reasons for this can be similarly explained as above. Interestingly, main roots of R. sinica on SS1-soil were horizontally extended and penetrated into the bottom of nearby outcrops (Fig. 5a and b). This may have resulted from shallow soils and weakly weathered bedrock (just below the soil layers). It is obvious that *R. sinica* on SS1-soil extracted deep water sources from the bottom of outcrops rather than from deeper layers below the soil laver. As discussed above, there was seepage of water from fissures in the outcrop that exhibited similar isotope values to spring water. It is reasonable to speculate that there was stable available water at the bottom of outcrops and plant root systems were shaped by the force of hydrotropism (Tsutsumi et al., 2003). Dawson and Pate (1996) described three different types of dimorphic root systems, our findings likely belong to a new type. Heilman et al. (2009) found that tree roots in karst woodland were restricted to surface soil; however, water sources for these trees were dominated by surface soil water. We further speculate that extracting deep water sources from the bottom of nearby outcrops was the main reason for the distribution of tree species on thin soils.

On March 23, 2009, isotope values for R. sinica on SS2-rock were more positive than those of potential water sources (Fig. 3b). R. sinica may have relied on water stored in cracks of the lower layers, which had been recharged by the rainfall (lack of samples) received between December 2008 and February 2009 (Fig. 2). As this time period was extremely dry and interspersed with scattered rain events, isotope values of the rain should be more positive than those sampled on March 2009. Although the rainfall amounts were small for each rain event in this time period, the amount of water infiltrated into cracks may be enlarged because most parts of the outcrop surface were impermeable (Chen et al., 2011). In addition, water stored in cracks of lower layers was more likely to avoid evaporation and the utilization by shallow-rooted species (Stothoff et al., 1999). Consequently, the relatively positive isotope values of *R. sinica* were correlated to low rainfall in the long dry time period. On Jul. 8, 2009, isotope values for R. sinica on SS2-rock were more negative than those for recent rainwater and plotted between previous rainwater and recent rainwater (or spring water, Fig. 4b). Iso-Source outputs showed that they used large proportions of recent and previous rainwater at the same time (Table 2). These results show that main water sources for R. sinica on SS2-rock shifted from water stored in rock fissures of the lower layers in the late dry season to water in the unsaturated zone in the early dry season. The reason for the shift of main water sources for R. sinica on SS2-rock could be similarly explained as those on SS1-rock.

According to the results of main water sources used by R. sinica on SS1-rock and SS2-rock, the major difference between tree species on continuous and isolated outcrops was that plants in the former habitat could effectively extract deep water sources in the late dry season, while plants in the latter habitat always relied on rainwater stored in rock fissures of the unsaturated zone. As water storage capacity of epikarst depends upon its thickness and continuity (Williams, 2008), the relatively small amount of water stored in isolated outcrops could not form a saturated zone, or at least it was not shallow enough to be reached easily by plant roots in the late dry season. Further more, in contrast to tree species on continuous outcrops, those on isolated outcrops more likely suffer from drought stress and the amount of water they consume by transpiration might decline in the late dry season.

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Contrary to the shifts of water sources used by *R. sinica* on rock outcrops and soils (at SS1), main water sources for A. trewioides on SS1-rock and SS2-rock was always dominated by water stored in rock fissures of upper layers, while the same plant on SS1-soil and SS2-soil always relied on shallow soil water. The constant utilization of shallow water sources by A. trewioides has a close relationship with its shallow root system. The advantages of shallow root system and the strategies for plants to adapt shallow root system have been discussed in our previous paper (Nie et al., 2011). It is obvious that there was competition between A. trewioides and R. sinica for shallow water sources in the early dry season, while the competition intensity decreased significantly when R. sinica switched to deeper water sources in the late dry season. Water source partitioning among species in the same habitat has been observed both in karst regions (Hubbert et al., 2001; Querejeta et al., 2007) and non-karst regions (Meinzer et al., 1999; Nippert and Knapp, 2007). Reduced competition between A. trewioides and R. sinica in the late dry season was one of the key factors for their coexistence in the same habitats.

### 5. Conclusions

In this study, we mainly used stable isotope techniques to determine water sources used by woody plants growing on dolomite outcrops (both continuous and isolated outcrops) and nearby soils in dry seasons. R. sinica (semideciduous tree) growing on continuous outcrops and nearby thin soils relied on deep water sources (ground water or water in the saturated zone, represented by spring water) in the late dry season. While it relied on rainwater stored in the unsaturated zone (for those growing on outcrop) or shallow soil water (for those growing on soils) in the early dry season. Remarkably, R. sinica growing on thin soils extracted deep water sources from the bottom of nearby outcrops rather than from deeper layers below the soil surface, as most of its main roots were horizontally extended and penetrated into the bottom of nearby outcrops. However, R. sinica growing on the isolated outcrop used no deep-water sources even during the late dry season. It relied on rainwater stored in rock fissures of the lower layers in the late dry season and used recent and previous rainwater simultaneously in the early dry season. In contrast, A. trewioides (deciduous shrub) growing on dolomite outcrops and nearby soils always relied on shallow water sources, which was a result of its shallow root system. Water source partitioning between tree and shrub species in the late dry season was one of the key factors for their coexistence in the same habitats. Results indicate that plant establishment could not be restricted by existence of rock outcrops when they are weathered to networks of cracks/fissures. Furthermore, the tree species (especially individuals growing on continuous outcrops) were more likely to extract deep water sources in the late dry season, which in turn led to normal transpiration in the late dry season. On the other hand, the utilization of deep water sources enhanced the water storage capacity of the study area. Further study is needed to measure the amount of plant transpiration by tree species, especially in the dry season, to assess their ecohydrological significance.

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