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THEMATIC ISSUE

Using stable isotopes to quantify water uptake by *Cyclobalanopsis glauca* in typical clusters of karst peaks in China

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Abstract In this study, stable isotopes (^{18}O and ^{2}H) were used to determine the sources of water for *Cyclobalanopsis glauca* in two habitats: limestone outcrops and thin soils in clusters of karst peaks in southwest China, where soils are scattered only in rock gaps and are underlain by rigid carbonate rocks. We used a direct inference approach and the IsoSource mixing model to estimate the contributions of different sources to the plant xylem water. The results showed that adult *C. glauca* growing on limestone outcrops mainly used water from soils in rock gaps, which comprised 65 % of all water sources during the rainy season, and water from rainwater sources during the rain-dry season and dry season; the proportion of deep water used increased during the dry season. In contrast, young *C. glauca* growing on limestone outcrops relied on a mixture of rainwater and soil water during the rainy and the rain-dry seasons, and on rainwater in the dry season, accounting for 66.3 and 64.0 % of water use, respectively. Adult *C. glauca* on thin soils mainly used soil water in rainy seasons, a mixture of soil water and rain water in the rain-dry season, and rainwater in the dry season. Water held within bedrock was essential for meeting plant transpiration requirements in karst peak cluster areas. The results will provide knowledge for the effective protection of vulnerable karst environments and for the recovery of vegetation on karst rocky desertification areas.

Keywords Water uptake · Stable isotopes · *Cyclobalanopsis glauca* · IsoSource · Epikarst water

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Introduction

Karst environments are characterized by shallow soils, exposed rocks, geological droughts, weak anti-jamming capacity, and poor stability (Cao et al. 2003), and are very different from other environments. Regional studies of water in karst aquifers are challenging, primarily owing to the highly heterogeneous characteristics of water circulation and the dramatic differences between karst and non-karst ecohydrology. Rainwater flows underground quickly and little is stored on the surface, which leads to the loss of surface water and desiccation (Deng et al. 2004). Water shortages and the great variability in water storage in time and space are often the key factors that control the biodiversity of natural karst ecosystems, which affect plant growth and hamper the restoration of vegetation. Because of the specific nature of the karst environment, and, in particular, the unique characteristics of water circulation and storage in karst areas (on the surface as well as underground), few species of plants are able to develop and survive in such difficult conditions.

The zones from which plant roots actively take up water have traditionally been difficult to determine. In bare karst

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areas, plant roots usually have large and expansive woody root systems that commonly penetrate deep into the rock fissures of the epikarst substratum. Plant roots are very difficult to excavate, but isotope approaches provide a direct and non-destructive method. White et al. (1985) applied stable isotopes to trace plant water resources and isotopic fractionation was not found in terrestrial plants, which is the basis of the theory for analyzing plant water sources using stable hydrogen and oxygen isotopes. From that point, stable ^{18}O and ^{2}H isotopes have been used extensively to determine the sources of plant water (Todd and James 1991; Brunel et al. 1995; Li et al. 2007; Nie et al. 2011; Deng et al. 2012). Todd and James (1991) used hydrogen isotope ratios to analyze the water sources of streamside trees and found that mature streamside trees growing in, or directly next to, a perennial stream used waters from deeper strata rather than surface stream water. In the Yucatan karst area, the isotope composition of soil water is expected to be different from that of groundwater at the peak of the dry season, as the aquifer is not subjected to evaporative enrichment (Socki et al. 2002). Unlike some semi-arid regions where the soil surface is often an important source of water vapor for plant leaves (Massman 1992; Wallace et al. 1993), karst terrains provide complex water sources for plant transpiration, which might include soil, epikarst water, and probably groundwater.

We hypothesized that natural individuals of woody tree species growing on carbonate rocks and thin soils over karst would tap the aquifer for a reliable supply of water. The naturally occurring concentration gradients in oxygen and hydrogen stable isotopes in rainwater, soil water, spring water, and stem water were used to investigate the sources of water used by *Cyclobalanopsis glauca*; the IsoSource mixing model was also used to estimate the contributions of the different sources to the plant xylem water. This research will provide knowledge for the effective protection of vulnerable karst environments and for the recovery of vegetation on rocky karst desertified areas.

Materials and methods

Study area

The study area is located in Nongla, near the town of Guling, in Mashan County, Guangxi, China (108 19 E, 23 29 N) (Deng et al. 2012). A subtropical monsoon climate dominates in this area. The mean annual rainfall, air temperature, and relative humidity are 1756.6 mm, 20 °C, and 85 %, respectively. Approximately, 82 % of the total rainfall occurs during the rainy season (between April and October). The main lithology is thick marl-silica dolomite from the middle Devonian Donggangling Formation with a gentle dip angle. The landscape is dominated by karst peak-cluster depressions, and consists of two flat depressions and 25 mountains with elevations of between 600 and 740 m. The difference in elevation between the depressions and the mountains is 120–260 m. The groundwater table (saturated zone) in the Fengcong depression area is heterogeneous and discontinuous. The groundwater table can be seen in sinkholes in the flat depression near the study site. The regional groundwater table is about 20 m beneath the depression (80 m beneath the mountains); and for this reason, it is not thought to play a significant role as a source of water for plants.

A perennial epikarst spring (named Landiantang Spring) located at the bottom of a hill slope that flows from the epikarst zone (−10 m in the recharge area to less than 0.1 m in the discharge area) through developed conduits. The catchment area of the spring may be larger than the 0.05 km^2 estimated from the topographic divide (Liu et al. 2007). The discharge varies from 0.01 to 20 l/s (Li et al. 2008). The spring water is the Ca–HCO_3 type. The watershed is a typical karst peak-cluster depression, and consists of several mountains. Hill slopes are characterized by steep slopes, shallow soils (5–10 cm deep and distributed only in carbonated gaps), and a high dolomite outcrop ratio (>90 %). Soils are well drained, gravelly, and calcareous. The initial infiltration rate and steady-state infiltration rate measured beneath the forest soil on the hillslopes are 0.1–1.25 mm min^{-1} and 0.01–0.55 mm min^{-1}, respectively (not published). This area experienced severe deforestation in the 1950s, and has been under natural restoration for almost 60 years. The main vegetation cover in the watershed is secondary forest, covering approximately 94 % of the total watershed area. *Cyclobalanopsis glauca* is the main species in the area, accompanied by *Cinnamomum burmannii BL.*, *Cmnamomum saxitilis H.W.Li*, and *Folium Clicis Latifoliae*. Tree roots can be seen in an excavated road near the study site. Tree roots are mainly distributed under the soil with 1 m, and up to 5 m deep.

Cyclobalanopsis glauca is the dominant species in the area, and is distributed from the top to the middle of the mountain (not found at the foot). *Cyclobalanopsis glauca* is an important and constructive native species in climax communities in karst ecosystems, and appears only in one certain grade during the karst forest restoration process. *Cyclobalanopsis glauca* is widely distributed throughout regions located between 23–34°N and 97–142°E, mainly in South China and subtropical Japan (Lou and Zhou 2001; Huang et al. 2009).

Sampling

Water samples were routinely collected at the Nongla Karst Dynamics Laboratory from July to December in 2008. Rain

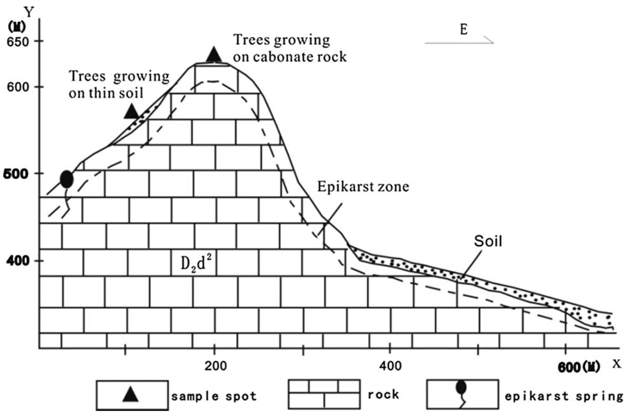

Fig. 1 Epikarst zone in the study area

water for single rain events was collected in a funnel attached to a plastic tank taking care to prevent evaporation (Li et al. 2007). A total of 23 rain events were monitored. Spring water was collected each month from July to December. Both rain water and spring water were stored in 15-mL capped plastic bottles, wrapped in parafilm, and frozen until stable isotope analysis.

Plants and soil were sampled simultaneously at two sampling sites on 9 July, 15 October, and 3 December in 2008. Samples of *C. glauca* growing on carbonate rock and thin soils (Fig. 1) were collected. Adult (DBH > 5 cm; $N = 4$) and young (DBH < 5 cm; $N = 3$) trees on carbonate rock were randomly selected, while only adult trees (DBH > 5 cm; $N = 5$) on thin soils were sampled. Suberized twigs were cut off. The bark of the twigs was peeled off and all leaves and green stem tissue were removed to avoid contamination of xylem water by isotopically enriched water (Ehleringer and Dawson 1992). Clipped twigs were 30–40 mm long; three to five twigs of non-green tissue were immediately placed in a 15-mL capped vial and wrapped in parafilm. There was soil at two of the sampling spots; at one, soil was distributed only in rock gaps, whereas at the other, there was a continuous cover of soil, about 10 cm thick. The soil was too thin to carry out stratified sampling. We decided to collect soil samples that were less than 5 cm deep to avoid strong evaporation. Soil samples weighing approximately 6–8 g, near with roots, were collected using a hand-driven probe, sealed in 15-mL capped vials, and wrapped in parafilm. Six soil samples were collected on each sampling occasion at each sampling site. After collection, twigs and soil samples were stored in the freezer (0–5 °C) until water extraction.

Water was extracted from soil and plant stem samples using a cryogenic vacuum distillation line (Li et al. 2007). The water content of the soil and plant stem samples was calculated as the difference in the weight of samples before and after thorough water extraction (100 °C, 3 h). Oxygen and hydrogen stable isotope analyses of plant stems and soil were conducted using an isotope ratio mass spectrometer (Finnigan MAT Delta V) at the Mass Spectrometer Laboratory, Chinese Academy of Forestry. D/H and $^{18}O/^{16}O$ ratios were also determined against the standard mean water (VSMOW, Vienna Standard Mean Ocean Water). The ^2H and ^{18}O content of precipitation and spring water were measured using an isotope ratio mass spectrometer (Finnigan MAT 253) at the Institute of Karst Geology, Chinese Academy of Geological Science. The ^{18}O content was determined with the H_2O–CO_2 equilibration method (Graig 1961), while the ^2H content was determined by the zinc reduction method (William et al. 1987; Coleman et al. 1982). Certified reference materials of water for stable hydrogen and oxygen isotopes of China were used as isotope standards, the precision of which against VSMOW was ±0.2 ‰ for $\delta^{18}O$ and ±1 ‰ for $\delta\ ^2H$. The overall analytical precision of the spectrometer was ±0.2 ‰ for $\delta^{18}O$ and ±2 ‰ for $\delta\ ^2H$. The isotopic composition of hydrogen or oxygen was expressed in delta notation (δ) as:

$$\delta D\left(\mathrm{or}^{18}O\right) = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1\right) \times 1{,}000,$$

where δ is the differential isotope value of the sample relative to the standard, and R is the absolute isotopic ratio (D/H or $^{18}O/^{16}O$) of the sample or standard.

Data analysis

To find the most probable sources of water uptake by *C. glauca* in our study, rainwater was treated as an individual potential water source, and was defined as that stored at a depth of 3 m in the shallow epikarst zone. Spring water was used to represent deep water sources about 10 m deep that are potentially accessed by plants (Bonacci 2001), the deepest depth of the epikarst zone in our study area.

The isotopic compositions of all potential water sources and xylem water were then input into the IsoSource model (Phillips and Gregg 2003). This model gives the distribution of the proportions of possible sources, and is based solely on isotopic mass balance constraints. We used a fractional increment of 0.1 in our calculations, and the uncertainty was no less than the outcome of the formula, 0.5 × increment × maximum differences between sources, to guarantee that no legitimate possible source combinations were missed (Phillips and Gregg 2003). We considered three distinct water sources (soil, shallow water, deep water) and used both $\delta^{18}O$ and δ^2H data for model calculations.

Statistical analysis was conducted using SPSS 13.0. analysis of variance to test for differences in the isotopic composition of spring water, soil water, and xylem water. The error line was the standard error (SE).

Results

Isotopic compositions of potential water sources and plant xylem water

The isotopic composition of precipitation was analyzed by plotting the monthly δ^2H values against the δ^{18}O values (Fig. 2). Because this relationship tends to be linear, it is usually analyzed by linear regression (Graig 1961). The best-fit line of this regression was the local meteoric water line (LMWL), which can be compared to the global meteoric water line (GMWL), the average of many LMWLs across the world (Graig 1961). There were strong seasonal variations in the oxygen isotopic composition of precipitation, with a range from −13.39 to −0.88 ‰. Correspondingly, the hydrogen isotopic composition of precipitation had a range from −97.1 to −9.95 ‰. Precipitation during the rainy season was significantly more depleted with respect to heavy isotopes when compared with the dry season because of the influence of the monsoon. The δ^{18}O values of precipitation were low in the rainy season, and had a mean value of −9.34 ‰. This is because the precipitation was controlled by tropical storms in summer. When the winter monsoon prevailed, the precipitation was controlled by the southwest warm and wet air mass and the cold front; δ^{18}O values were high, and the mean value was −4.64 ‰. The δ^{18}O in precipitation is controlled by the type of monsoon, its source, and

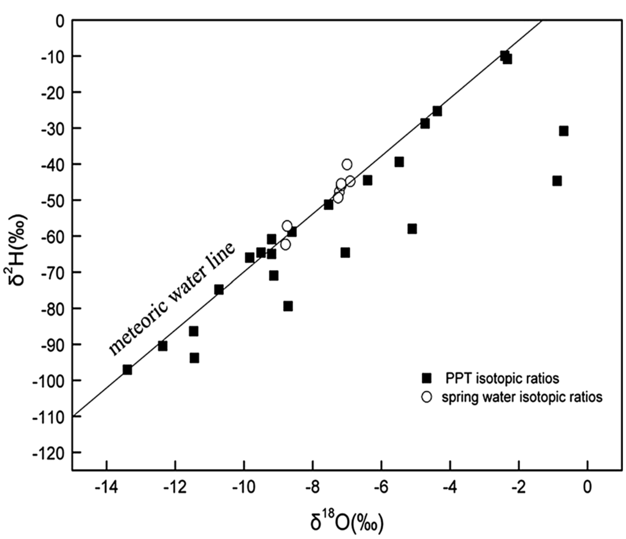

Fig. 2 The relationship between δ^2H and δ^{18}O in rainwater and spring water in the study area. The fitted line is the global meteoric water line ($\delta^2H = 8\ \delta^{18}O + 10$; Graig 1961)

characteristics (Li et al. 2000). Figure 2 also shows that several precipitation samples deviate from the GMWL because samples may have been affected by local precipitation, or because of the dry atmosphere in the dry season and low precipitation (<3 mm). ^{18}O enrichment was due to the strong evaporation.

Stable isotopes in epikarst spring water are also shown in Fig. 2. Epikarst spring water δ^{18}O values varied between −7 and −8.79 ‰, and δD values were between −40.1 and −62.3 ‰. δ^{18} O and δ^2H signatures of epikarst spring water were plotted along the meteoric water line, and deviated to the right of the GMWL, as shown in Fig. 2; this is evidence that the spring water supply source in this area came from precipitation. Rainwater circulated in the lower epikarst zone and there were no strong interactions between water and carbonate rock.

Figure 3 shows the distribution of δ^{18}O and δ^2H for plants and their corresponding potential water sources in different seasons. The average δ^2H content of gap soil increased from −95.09 ‰ in the rainy season to −64.74 ‰ in the rainy-dry season, and approached −35.63 ‰ in the dry season. Average δ^{18}O values in gap soils varied from −11.8 to −9.39 ‰ in the rainy season to −6.61 ‰ in the rainy-dry season, and then reached −2.00 ‰ in the dry season. The average δ^2H values in thin soils were −74.88 ‰ in the rainy season, −82.43 ‰ in the rainy-dry season, and −62.78 ‰ in the dry season. This peculiar pattern is due to the large amount of rainfall during the sampling period, which probably diminished the soil evaporative enrichment process in the rainy season (Li et al. 2006). The stable isotope content in the gap soil was richer than that in thin soil during the dry season. Previous studies have reported more intense evaporation in bare karst regions than in covered karst areas (Deng et al. 2004).

Figure 3a, b, and c shows that stable isotope values for adult *C. glauca* growing on limestone rock ranged from −102.63 to −61.73 ‰ for δ^2H, and from −13.37 to −6.89 ‰ for δ^{18}O. δ^2H values for young growing on limestone rock ranged from −98.57 to −61.73 ‰ for δ^2H, and from −13.37 to −8.33 ‰ for δ^{18}O. Stable isotope values for adult *C. glauca* growing on thin soils had a range from −83.81 to −53.58 ‰ for δ^2H, and from −11.75 to −5.34 ‰ for δ^{18}O.

IsoSource estimation of possible contributions of potential water sources

Tables 1, 2, and 3 show the proportions of possible water sources for *C. glauca* for the two habitats during the rainy, rainy-dry, and dry seasons, respectively.

Adult *C. glauca* growing on limestone outcrops mainly used soil water sources, even though there was only gap soil, and accounted for 65 % of all water sources, and 29 %

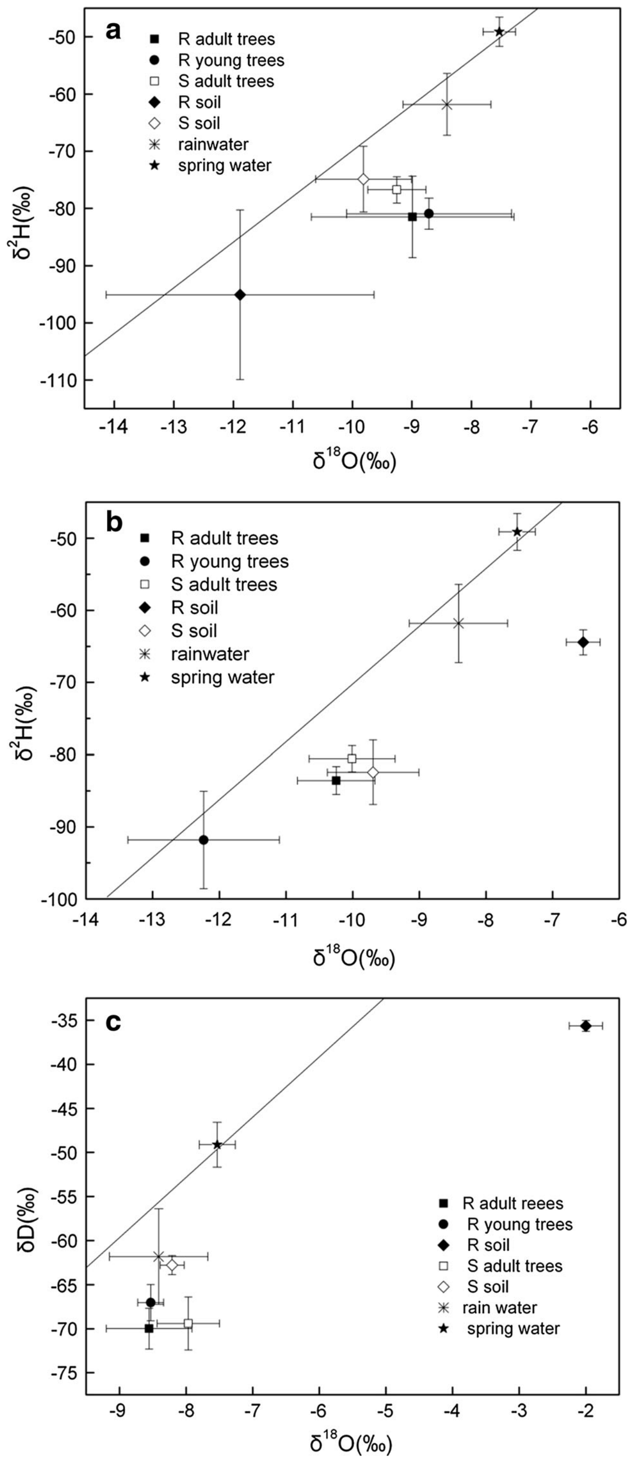

Fig. 3 The relationship between δ^2H and δ^{18}O (mean ± SE) in stem water of *C. glauca* growing on two habitats (R-growing on limestone rock; S-growing in thin soil) in southwest China and their potential water sources (precipitation, soil water, and spring water). Each point represents the average isotopic content. The *Fitted line* is the meteoric water line ($\delta^2H = 8\,\delta^{18}O + 10$; Graig 1961) (**a** rainy season; **b** rainy-dry season; **c** dry season)

Table 1 Mean contributions of possible water sources (%) for *Cyclobalanopsis glauca* in the two habitats during the rainy season (minimum–maximum in brackets)

	Adult trees in limestone rock	Young trees in limestone rock	Adult trees in the soil
Rain (july 8)[a]	15 (6–24)	27 (18–36)	8 (1–15)
Rain (july 9)[a]	14 (3–25)	16 (5–27)	16 (6–26)
Soil[b]	65.5 (64–67)	45.5 (44–47)	64 (61–67)
Spring water (july)[c]	5.5 (5–6)	11.5 (11–12)	12 (12–12)

Water source contributions were calculated using the IsoSource model (Phillips and Gregg 2003)

[a] Date in brackets indicates that rainwater was sampled on that day

[b] Soil isotopic values represent average values

[c] Spring water isotopic values represent average values of sample month

Table 2 Mean contributions of possible water sources (%) for *Cyclobalanopsis glauca* in the two habitats during the rainy-dry season (minimum–maximum in brackets)

	Adult trees in limestone rock	Young trees in limestone rock	Adult trees in the soil
Rain (september 25)[a]	73.2 (71–75)	20.2 (18–22)	47 (45–50)
Rain (september 27)[a]	5.5 (0–18)	4.8 (0–15)	7.1 (0–22)
Soil[b]	11 (7–16)	66.3 (62–71)	31.1 (29-33)
Spring water (august)[c]	5.1 (0–16)	4.2 (0–14)	7.3 (1–20)
Spring water (september)[c]	5.2 (0–17)	4.5 (0–15)	7.6 (0–19)

Water source contributions were calculated using the IsoSource model (Phillips and Gregg 2003)

[a] Date in brackets indicates that rainwater was sampled on that day

[b] Soil isotopic values represent average values

[c] Spring water isotopic values represent average values of sample month

Table 3 Mean contributions of possible water sources (%) for *Cyclobalanopsis glauca* in the two habitats during the dry season (minimum–maximum in brackets)

	Adult trees in limestone rock	Young trees in limestone rock	Adult trees in the soil
Rain (november 2)[a]	4.3 (2–8)	0 (0–0)	2.7 (0–4)
Rain (november 3)[a]	56 (55–58)	64 (63–65)	56.3 (48–65)
Soil[b]	4 (0–8)	1.5 (0–3)	19.5 (0–44)
Spring water (september)[c]	15.6 (5–33)	12.5 (4–21)	10.7 (2–35)
Spring water (october)[c]	20.1 (4–30)	22 (14–30)	10.8 (0–26)

Water source contributions were calculated using the IsoSource model (Phillips and Gregg 2003)

[a] Date in brackets indicates that rainwater was sampled on that day

[b] Soil isotopic values represent average values

[c] Spring water isotopic values represent average values of sample month

of water came from rainwater during rainy season. In the rain-dry season, soil water accounted for 11 % of the water source, while rainwater accounted for 78.7 %. Adult *C. glauca* still used a large proportion of rainwater sources (60.3 % in total), but the proportion of deep water increased from 5.5 % in the rainy season to 35.7 % in the dry season.

In the same habitats, young *C. glauca* had different water uptake patterns. They relied on a mixture of rainwater and soil water during the rainy season (43 and 45.5 %, respectively), and switched to soil water (66.3 % in total) in the rain-dry season, then returned to use rainwater in the dry season.

Adult *C. glauca* on thin soils mainly used soil water in the rainy season, a mixture of soil water and rain water in the rain-dry season, and rainwater in the dry season. The proportion of soil water uptake by adult *C. glauca* growing on thin soils declined slowly, from 64 % in the rainy season, to 31.1 % in the rain-dry season, and further to 19.5 % in the dry season. The proportion of rain water and spring water increased as rain declined. The proportion of rain water increased from 24 % in the rainy season, to 54.1 % in the rain-dry season, and to 59 % in the dry season. The proportion of spring water increased from 12 % in the rainy season, to 15 % in the rain-dry season, and to 21.5 % in the dry season.

Discussion

Spatial heterogeneity in the karst peak cluster area in southwest China was very high, so the light intensity, air humidity, and soil temperature varied even though it only covered a small area (Deng et al. 2004). *Cyclobalanopsis glauca* in different habitats could suffer varying degrees of drought stress, and could adapt to the karst drought stress, not only by adjusting water use patterns, but also by developing some drought-resistant structures on leaves, such as column-shaped palisade cells and a tighter arrangement of sponge cells and hyperdermis (Deng et al. 2004).

According to initial expectations, all the investigated trees partly used epikarst water, and the proportion used increased as rain decreased. The IsoSource outputs (Table 3) showed that adult *C. glauca* growing on limestone outcrops mainly used epikarst water (shallow water and deep water accounted for 60.3 and 35.7 % of water uptake, respectively) during the dry season. In our study area, the roots of *C. glauca* were very long. All studied trees were standing on rock crevices, which meant that their roots were growing into the rock fissures. Some roots grew down along the fissures, some of which protruded out of the rock onto the slope. Field observations confirmed that it was possible for *C. glauca* to tap deep water, depending on morphology. Huang et al. (2011) used Granier's sap-flow method to measure the transpiration from *C. glauca* standing on rocky hilly slopes in south China and found that the stand transpiration and canopy stomatal conductance were high even during the dry season in the karst region which may result from that karst plants obtained water partially from the epikarst zone. Our research confirmed the hypothesis of Huang et al. (2011). Some studies also showed that phreatophytic species typically exhibit xylem sap isotopic values closely matching those of aquifer water, particularly during drought periods when other sources of water are scarce or depleted (Zencich et al. 2002; Chimner and Cooper 2004; Nie et al. 2011; Deng et al. 2012). Deng et al. (2012) reported that water storage in the epikarst zone is an essential water source for trees growing on carbonate rocks in Southwest China. Nie et al. (2011) also found that the deciduous tree species *Radermachera sinica* mainly used deep water sources during the dry season, and a mixture of rainwater and deep water sources during the wet season (Nie et al. 2011). Conversely, Jose et al. (2007) reported that native trees growing on shallow karst soils in the northern Yucatan used little or no groundwater and depended mostly on water stored within the upper 2–3 m of the soil/bedrock profile.

Most previous studies have used direct or indirect methods, such as stable isotopes (Deng et al. 2012), root excavation (Jose et al. 2007), or Granier's sap-flow method (Huang et al. 2011) to confirm that karst plants obtain water partially from the epikarst zone. To plan for epikarst water resources, it is important to know the depth of water uptake. The epikarst is the uppermost layer of a karstified rock in which a large proportion of the fissures has been enlarged by chemical erosion. An epikarst allows rapid infiltration and storage of large quantities of water. At this research site, the mean annual rainfall is 1,756.6 mm, and rainfall mainly occurs between June and October. Abundant rainfall and high temperatures in the same period provide advantageous conditions for the development of an epikarst zone, therefore, the epikarst is usually several meters thick; at its deepest it is up to 10 m in this area (Jiang et al. 1999). Combining the IsoSource outputs with the root depth observations, *C. glauca* in the two habitats, including adult and young trees, mainly used soil water and water stored in the shallow epikarst zone through the seasons studied, and accessed deep water in the dry season. Oliveira et al. (2005) reported extensive depletion of soil water stored at depths of 4–7.5 m by trees in a Brazilian dry tropical forest during dry periods. Nepstad et al. (1994) and Jipp et al. (1998) also observed substantial tree water uptake at depths exceeding 8 m in an evergreen tropical forest in eastern Amazonia. The results imply that more attention should be directed to plant water sources, because spring water is a vital source of water for local people in this karst peak cluster area in southwest China (Qin et al. 2005). There is, however, intensive use of spring water by trees during droughts, which aggravates the water crisis in the karst area.

Conclusions

Cyclobalanopsis glauca mainly used soil water and water stored in the shallow epikarst zone in the seasons studied, and touched on deep water during the dry season. This research will provide knowledge that will support the efficient protection of vulnerable karst environments and promote the recovery of vegetation on desertified rocky karst areas.

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