Water source segregation along successional stages in a degraded karst region of subtropical China

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Running title: Water source segregation at a community level

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Abstract

Questions: Water source segregation among plant species has been widely observed in a variety of ecosystems. However, whether correlated segregation occurs at a community level and its relationship to successional stages has rarely been studied.

Location: Open shrubland, dense shrubland and secondary forest on adjacent rocky karst hill slopes, Southwest China.

Methods: Plant samples of the five most dominant species from each community type were collected seven times throughout the growing season. Dynamics of stem water isotope values (SWIVs) for the five species within each community were examined using a regression analysis of the averaged isotope value of each species versus sampling time. Overall variation of SWIVs was compared among communities. Similarities between communities were further distinguished by comparing the dynamics of SWIVs for the species shared by different communities and studying their relationship to rainwater.

Results: The fitted regression line for each community was statistically significant ($P < 0.05$) with all species falling between the upper and lower limit lines of the 95% prediction interval of stem water isotopic composition; these convergent dynamics point toward a similar water source for different species within each community. The common species from the secondary forest exhibited fewer fluctuations in SWIVs than those from the open shrubland, which was consistent with the significantly smaller overall variation in SWIVs for the secondary forest. As smoother dynamic of SWIVs over time is related to using rainwater received over a longer period of time, the secondary forest was estimated less dependent on rainwater received within short time spans than the other two communities.

Conclusions: Our study revealed community-level water source segregation in a karst region. Early successional plant communities were more dependent on recent rainwater received within short time spans and thus were potentially more vulnerable to reduced rainfall frequency than late successional stage communities.

Keywords: stable isotope, plant water source, plant community, successional stage, rocky environment, common species, karst region, subtropical China

Introduction

There is a growing interest in evaluating and understanding the responses of plant communities to climate changes, especially to the change in precipitation (de Dios Miranda, Padilla, & Pugnaire, 2009; Pérez-Ramos, Rodríguez-Calcerrada, Ourcival, & Rambal, 2012). In order to provide substantial support for achieving these goals, sources of water used by plants need to be known (Asbjornsen et al., 2011). Water source segregation has been widely reported at a species level in different ecosystems (Silvertown, Araya, & Gowing, 2015 and references therein), however, correlated differences among communities have rarely been studied despite the leading role they play in shaping community dynamics under precipitation change (Zhang, Niinemets, Sheffield, & Lichstein, 2018). Moreover, in regions where shallow soil is underlain by weathered bedrock, knowledge of plant water source is far less advanced because of the complex substrate conditions (Cardella Dammeyer, Schwinning, Schwartz, & Moore, 2016).

Karst landscapes, which occupy 10% to 15% of the total continental area (Ford & Williams, 1989), are typical examples of a region with complex substrates. Most weathered materials that are derived from soluble carbonate bedrock (such as limestone and dolomite) can dissolve in water and result in an extremely slow rate of soil formation and a shallow soil coverage (Cao, Yuan, & Zhang, 2004). Additionally, this thin soil layer is usually underlain by weathered bedrock, which is manifested by networks of cracks and crevices. In these cases, most rainwater will likely penetrate rapidly through the thin soil layer then flow along connected rock fissures into deeper layers such as the temporary saturated zone or into the deep groundwater (Cao et al., 2004). A small part of rainwater can also be stored in tightly closed fissures within the unsaturated zone (Estrada-Medina, Graham, Allen, Jiménez-Osornio, & Robles-Casolco, 2013). Although, sources of water used by plants growing in karst regions are always hard to identify, related results have become more abundant during the last 10 years (Nie, Chen, Wang, & Schwinning, 2017 and references therein). Almost all of these studies indicate that some karst species generally rely on rainwater stored in the unsaturated zone (Elkington et al., 2014; Heilman, McInnes, Kjelgaard, Owens, & Schwinning, 2009; Querejeta, Estrada-Medina, Allen, & Jiménez-Osornio, 2007), while other species use larger proportions of temporally stable water sources (such as water in the temporary saturated zone or groundwater) from greater depths (McCole & Stern, 2007; Nie, Chen, Wang, & Yang, 2012; Rong, Chen, Chen, Wang, & Du, 2011). Significant results have been achieved at a species level; however, whether water source segregation occurs at a community level in karst regions has rarely been tested.

Although there is a lack of direct evidence, the obvious differences among communities at various successional stages in karst regions point to the probable existence of plant water source segregation at a community level. Generally, the microenvironment in early successional sites is warmer and drier than in late successional sites or mature forests (Batalha, Pipenbaher, Bakan, Kaligarič, & Škornik, 2015; Pineda-Garcia, Paz, & Meinzer, 2013). Thus, especially in water-limited regions, early successional communities are likely to have more species that grow in harsher soil conditions than late successional ones (Batalha et al., 2015) with 'escape' from drought being the main strategy used to deal with drought stress (Delzon, 2015). Additionally, early successional community species normally have greater hydraulic conductivity than late successional community species (Markesteijn, Poorter, Paz, Sack, & Bongers, 2011; Zhu, Song, Li, & Ye, 2013), which helps these species capture even small pulses of rainfall. Furthermore, early successional communities are normally dominated by grass or pioneer shrub species (Batalha et al., 2015; Nie, Ding, Zhang, & Chen, 2018). Therefore, the main water sources used by the dominant species of one community are expected to converge to a similar type and differ from that of communities at other successional stages.

Few methods are available for efficiently identifying plant water source at a community level. One possible method is based on the isotope ratios of atmospheric water vapor and the related liquid water (e.g., soil water at different depths); however, the discrepancy between the isotope ratio of plant xylem water and transpired water, which results in a non-steady state, complicates water source identification (Griffis, 2013; Lai, Ehleringer, & Bond, 2006). Moreover, the performance of this method requires a relatively uniform underlying surface, which is not found in fragmented karst landforms. Since roots of most plants (except some halophytic and xerophytic species) do not fractionate water during uptake and the evaporation from suberized stems is negligible (except Vargas, Schaffer, Yuhong, & Sternberg, 2017), the isotope ratios of water in suberized stems reflect the uptake-weighted average isotope ratio of water in the root zone (Ehleringer & Dawson, 1992), and can be used to differentiate plant water sources (reviewed by Rothfuss & Javaux, 2017). Additionally, in scenarios where potential water sources within the root zone are hard to locate and sample, the dynamics associated with the isotope ratios of stem water and its relationship to that of rainfall can also provide information on plant water source. For example, based on the shifts in stem water isotope ratios after rainfall, together with the recovery of stem water potential, Schwinning (2008) concluded that tree species of the Edwards Plateau Karst did not commonly reduce aquifer recharge by tapping directly into perched water tables. In the light of these studies, it appears that the probable existence of plant water source segregation at a community level in karst regions can also be revealed by a similar approach.

In order to reveal the probable existence of plant water source segregation at a community level, three community types (open shrubland, dense shrubland and secondary forest) typical to a degraded karst region of southwest China were studied. Knowledge about plant water sources segregation is critically important to this region since water shortage is the primary limiting factor for vegetation restoration (Jiang, Lian, & Qin, 2014). Suberized stems for the top five dominant species of each community (which were used to represent the integrated condition of each community) were sampled monthly from May to December 2014. Rainwater samples were collected for each rain event throughout the year. The primary goal of this study was to determine (1) whether main water sources used by dominant species within a community converged to a similar pattern, and (2) whether water source segregation at a community level can be detected and related to communities at different successional stages.

Materials and Methods

Site description

The study site is a small watershed located in Huanjiang Country, Northwest Guangxi, Southwest China (24°57′–24°58′N, 107°58′–107°59′E). This region experiences a typical subtropical mountainous monsoon climate, with annual rainfall of 1389 mm and annual air temperature of 19°C. The wet season occurs between the end of April and early September, during which more than 70% of the total annual rainfall is received. The watershed is on the edge of a continuously distributed karstic peak-cluster depression area, and is dominated by a series of isolated hill slopes scattered around a flat depression (Fig. 1). The soils in the depression are often deep and clayey. Hill slopes are steep (usually exceed 25°) and characterized by an extremely high limestone outcrop ratio (80-95%), soils are only found in some microhabitats (such as “rock pan” and “rock groove”) near surface or belowground rock fractures. Limestone outcrops on hill slopes are not continuously distributed and are characterized by unordered gaps, fractures and crevices (Fig. 1). In this circumstance, most rainfall quickly infiltrates belowground and rarely generates surface runoff.

Figure 1

Southwest China experienced severe deforestation from the 1950s through the mid-1980s due to human disturbances and resulted in degraded land and sparse vegetation cover. Currently, shrub-grassland and shrubland are the most common vegetation types in the region; secondary forests are normally limited to natural reserves, and local “Fengshui Forests” on hill slopes close to villages, places of worship that protect a village. Moreover, shrub vegetation can further be distinguished into two types, open shrubland (OS) and dense shrubland (DS).

Three plots for each type of shrubland, as well as for secondary forest (SF) were established (nine plots in total) in June 2013. Shrubland plots were 10 m × 10 m, secondary forest plots were 30 m × 20 m. Experimental plots were at least 50 m apart from each other. The SF located in the middle and was about 800 m and 1200 m apart from the OS and DS, respectively. Vegetation investigation was carried out during the peak season (July and August) of plant growth in the study region. Nomenclature follows Flora of China (Flora of China Editorial Committee, 1994). Site characteristics are listed in Table S1.

Sampling

Rainwater samples were collected for each rain event throughout 2014. Rainwater samples were placed in capped vials, wrapped in parafilm and stored in a freezer. The temporal distribution of rainfall data was recorded at a meteorological station located in the small catchment. As some species are likely to have deep roots and access the deep-water pool that buffers the isotopic effects of a single rainfall event, phreatic groundwater was sampled (from local spring seepage) to represent water from this pool by following previous studies in karst regions (McCole & Stern, 2007; Nie et al., 2011; Schwinning, 2008).

Plant samples were collected seven times (May 28, Jul. 5, Aug. 9, Sep. 4, Sep. 28, Oct. 25, and Dec. 2) throughout the growing season of 2014. At each sampling time, suberized twig (three to five replications per species) samples for the five most dominant species of each community type (Table S1) were cut from the sunny side of the slope. To avoid contamination of xylem water by isotopically enriched water, all leaves and green stem tissues were removed (Schwinning, 2008). Clipped twigs were immediately placed in a capped vial, wrapped in parafilm and placed in a cooler with ice for transportation to the laboratory. In the laboratory, samples were stored -30°C. Moreover, mature leaves from each twig obtained for plant water source identification were collected for further analysis. Each sampling campaign was completed within two clear days. During early October, a tree species (*Sapium rotundifolium*) from the SF began to lose leaves, thus twig and leaves were not sampled for this species during the last two sampling times.

Laboratory analysis

Stem water was extracted by the cryogenic vacuum method described by Ehleringer, Roden, & Dawson (2000). Stem water and rainwater samples were measured by using isotope ratio infrared spectroscopy (IRIS, DLT-100, Los Gatos Research, Mountain View, CA, USA). Isotope ratios are reported in delta notation in units of per mil (‰):

$$\delta D \text{ (or } \delta^{18}O) = (R_{sample} / R_{standard} - 1) \times 1000 \qquad (1)$$

Where, δD and $\delta^{18}O$ refer to the stable hydrogen and oxygen isotope values for the sample, respectively; R_{sample} and R_{VSMOW} are the absolute isotopic ratio (D/H or $^{18}O/^{16}O$) for the sample and standard (VSMOW, Vienna Standard Mean Ocean Water), respectively.

Local meteoric water line (LMWL) was generated based on the δD and $\delta^{18}O$ values of each rain event throughout the study year. Deviation of individual point (each rain event or each stem water sample) from the LMWL was expressed by a line-conditioned excess (LC-excess). LC-excess was calculated based on a formula proposed by Landwehr & Coplen (2006):

$$\text{LC-excess} = \delta D - a \times \delta^{18}O - b \qquad (2)$$

Where a and b are the slope and y-intercept, respectively, of the LMWL. Precipitation itself can also deviate from the LMWL, and thus have either positive or negative LC-excess (Allen, Kirchner, & Goldsmith, 2018). Soil water and plant stem water samples normally have negative LC-excess values, a more negative LC-excess values of stem water sample suggests that source water has experienced stronger evaporative enrichment before it was absorbed by plant roots (Landwehr & Coplen, 2006; Sprenger, Leistert, Gimbel, & Weiler, 2016).

The chlorophyll concentration in leaves was measured with a chlorophyll meter (Konica-Minolta Holdings, Inc., SPAD-502Plus). We calculated specific leaf area (SLA) as the ratio of leaf area to dry mass. Approximately 30 - 50 fresh clean leaves were randomly selected from each sample bag and measured by LI-3100 (LiCor, Lincoln, Nebraska, USA) to determine leaf area. These leaves were subsequently oven dried at 75℃ to constant weight. The total mass of all the dried leaves was measured and divided by the total leaf number to determine the average leaf dry mass per sample. Leaf water content (LWC) was calculated as LWC = (FM-DM)/FM, where FM and DM represent fresh mass and dry mass of the leaves, respectively.

Data analysis

All data were initially tested and log-transformed when necessary to fulfill statistical assumptions. Stem water isotope values of all samples collected within the same community were first integrated into a data set, with the averaged stem water isotope value of each species acting as a replicate and all replicates from the same sampling time forming a group. Moreover, the seven sampling times were numbered from 1 to 7 according to the precedence order. Then, the regression analysis between averaged stem water isotope values and numbered sampling times for one community, with the upper and lower limit lines of 95% prediction interval, can be performed. Convergence of water source utilization by the dominant species of one community to a similar pattern was then investigated based on how the averaged stem water isotope values plotted between the upper and lower limit lines.

In order to detect whether plant water source discrepancy occurs at a community level, the overall features, such as the range (maximum - minimum), standard deviation (SD) and coefficient of variance (CV) of stem water isotope values of the dominant species over time were compared among communities. The reasons for doing this include: (1) isotope values of precipitation in this region varies widely throughout time, so the stem water isotope value of species that rely on shallow water sources (which are frequently recharged by recent precipitation) should exhibit relatively large variation; while (2) deep water sources (such as water in the saturated zone or groundwater) in karst regions normally buffer the variation of isotope values of rainfall (McCole & Stern, 2007; Nie et al., 2011), the stem water isotope value of species that utilize deep water sources should exhibit smaller variation. In practice, for example, all (for the seven sampling times) stem water isotope values of one species from the same community were used to calculate the range of this species, and then the ranges of all the selected species from the same community were compared with that of other communities as a group. One-way analysis of variance (ANOVA) was used to determine significant differences among communities. One-way analysis of variance was further used to determine significant difference in stem water isotope values at each sampling time. For each leaf trait, the difference between the shared dominant species was determined by treating each sampling date as a replicate. Statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and OriginPro 8 (OriginLab, Hampton, MA, USA).

Results

Rainfall distribution and isotopic compositions of rainwater and plant stem water

Figure 2 shows the rainfall measured in the study area and the fluctuation of δD values of rainwater. In 2014, about 1570 mm of rainfall was received in total, which was about 13% more than the multi-year average value (1389 mm). About 75% of the total precipitation was received during the wet season (April – September). All plant sampling was conducted on clear days. There was no rainfall within the last 25 days before the sixth sampling time (Oct. 25), water in the environment probably experienced evaporative enrichment during this period.

Figure 2

Stable hydrogen isotope values of rainwater varied widely throughout this year (between -118.3‰ and 32.3‰) and showed a general seasonal pattern, with more negative values in the wet season and more positive values in the dry season (Fig. 2). Specifically, δD values of rainwater received before the first sampling time were more positive than -25.0‰. LC-excess of rainfall exhibited similar fluctuation pattern as rainfall isotope values, but much more concentrate around the value of zero (Fig. 2).

The local meteoric water line (LWML) determined from local rainfall samples was indistinguishable from the global meteoric water line (Fig. S1). Moreover, stem water sample values fell along rather than below the LWML (Fig. S1), indicating that rainwater didn't undergo strong evaporative enrichment before being absorbed by plant roots. In accordance with this speculation, for most of the sampling times (except the sixth sampling time), averaged stem water LC-excess values of the dominant species from each community was either less negative than -10‰ or marginally within the range of the LC-excess values of rainfall received during the past month prior to each sampling time (Table S2). At the sixth sampling time, LC-excess values of stem water were much more negative than that of rainfall (Table S2), indicating the use of evaporatively enriched water sources after 25 days without rainfall (Fig. 2).

Convergent dynamics of stem water isotope values within a community

Stem water isotope values of dominant species from the three communities showed similar seasonal patterns, decreasing sharply from the early to the middle wet season then increasing gradually during the dry season (Fig. 3). Specifically, the relationship between the numbered sampling times and all stem water isotope values from each of the three communities could be described by a second-degree polynomial regression line (Fig. 3), and the F-tests at the 0.05 level indicated that these regression lines were statistically significant. Moreover, the upper and lower 95% prediction lines of each regression line were generated. Stem water isotope values of most (except for a few plant individuals) dominant species from the same community fell within a similar zone formed by the same prediction lines (Fig. 3), indicating that water source utilization by the dominant species from the same community converged to a similar pattern.

Figure 3

As shown in Table 1, there was no significant difference between averaged stem water isotope values among different communities. However, the variation in stem water isotope values differed significantly among communities (Table 1). Specifically, the range and SD of stem water isotope values of the SF were significantly smaller than that of the shrubland. CV of the stem water isotope values of the SF was also significantly different from that of the DS, and the difference between the SF and the OS was marginally significant (*P* = 0.072).

Table 1

Divergence water use patterns among the shared dominant species of different communities

At the first sampling time, stem water isotope values of the shared deciduous tree species (*S. rotundifolium*) from the SF (-30.8‰) was significantly more negative (*P* < 0.01) than those from the OS (-17.2‰, Fig. 4a), indicating that they derived most water from isotopically different sources of water. Since, the isotope values of all rainfall received before the first sampling time (about 500 mm within 5 months) were more positive than -25‰ (Fig. 2), we can conclude that the tree in the SF relied on water from a deep water pool that was represented by phreatic groundwater, while the same species in the OS relied on rainwater received prior to the first sampling time. During the following period, stem water isotope values of the tree gradually decreased to below that of the phreatic groundwater on Sep. 28, which matched the main trend of isotope values of rainfall received within the same period (Fig. 1, Fig. 4a). On the other hand, stem water isotope values of the tree from the SF always plotted closer to the phreatic groundwater line than those associated with the OS, indicating that the tree utilized larger proportion of phreatic groundwater in the SF.

Figure 4

Stem water isotope values of the shared evergreen shrub species (*R. kwangsiensis*) from the SF and OS exhibited similar fluctuation patterns throughout the study period (Fig. 4b) and also followed the dynamic trend of rainfall (Fig. 2), indicating that the shrub species in both communities relied on rainwater received prior to each sampling time. On the other hand, stem water isotope values of the shrub from the SF always plotted above that from the OS and experienced less variation throughout the study period (Fig. 4b), indicating the existence of isotopic differences between the rainwater used by the shrub associated with different communities. As isotope value of a mixture of rainwater normally becomes more stable with more rainfall events are involved; the less variation of stem water isotope values for the shared shrub from the SF indicated the utilization of a mixture of rainwater that was received over a longer period (compared with the shared shrub from the OS) prior to each sampling time.

In accordance with the differences in the main water source utilized by the same species from different communities, these species differed in water-related leaf function traits. As shown in Fig. S2, there was no significant difference in leaf area, however, leaf water content and chlorophyll SPAD value were significantly higher for the same species in the SF. The deciduous tree also exhibited significantly higher specific leaf area in the SF, while the evergreen shrub had no significant difference between communities.

Discussion

Our results showed that isotope values for the stem water samples collected on a monthly basis plotted along rather than below the LMWL, which differs from most of the related karst studies (Estrada-Medina et al., 2013; McCole & Stern, 2007; Rong et al., 2011) and non-karst studies (Song, Zhu, Li, & Yu, 2014; Wei, Lockington, Poh, Gasparon, & Lovelock, 2013). In these studies, all stem water samples plotted below the related LMWL, indicating that the plants derived water from sources that had been evaporatively enriched (compared with rainfall input). The key reason for the consistent results is that surface and subsurface soil water inevitably experiences evaporation and, thus, evaporative enrichment in common conditions; deeper soil layers usually receive water from upper layers because of water replacement (Asbjornsen et al., 2011). In this study, community type plots are located on rocky hill slopes where the earth surface is covered by fractured bedrock (Fig. 1) and rainwater normally flows below ground along twisted fractures and cracks. In this case, rainwater stored in underground karst features should have experienced very slight evaporative enrichment (Nie et al., 2011; Querejeta et al., 2007). Further evidence for this view comes from data about LC-excess. In this study, for three out of the seven sampling times, averaged stem water LC-excess values of the dominant species from each community was less negative than -10‰ (Table S2). This value is equivalent to mobile soil water and/or groundwater that normally undergo slight evaporative enrichment (Hasselquist, Benegas, Roupsard, Malmer, & Iistedt, 2018; Tiemuerbieke et al., 2018). Precipitation itself can also have either positive or negative LC-excess (Allen, Kirchner, & Goldsmith, 2018), for example, the most negative LC-excess of rainfall received during the study period was around -20‰ (Table S2). In this circumstance, except for the sixth sampling time (on Oct. 25), the relatively more negative averaged stem water LC-excess of the rest sampling times does not necessarily mean rainwater has undergone strong evaporative enrichment before being absorbed by plant roots. Therefore, isotope values of stem water can be compared directly with that of rainwater received prior to each sampling time. Taking the highly dynamic nature of rainfall isotope values into consideration (Fig. 2), we can predict that the fewer fluctuations of stem water isotope values throughout time is associated with the utilization of a mixture of rainwater received within a longer period prior to each sampling time.

The convergent dynamics of stem water isotope values of dominant species from the same community indicates the similarity of main water sources used by the various species within a community, which contradicts the general idea of niche segregation among co-existing plants (Lundholm, 2009; Silvertown, 2004). Significant hydrological niche segregation has been found in field studies across vegetation types ranging from arid to wet, with most of these studies providing evidence that water source segregation among different species was occurring (Silvertown et al., 2015 and references therein). Moreover, although there is a lack of substantial evidence that species coexistence depends upon the presence of niche segregation, water source segregation has long been treated as one of the most popular mechanisms for reducing competition for water (Moreno-Gutiérrez, Dawson, Nicolás, & Querejeta, 2012; Palacio, Montserrat‐Martí, & Ferrio, 2017). However, on karst rocky hill slopes, encroachment of plants depends upon the presence of rock fractures and all plant individuals being rooted directly into these fractures (Fig. 1). Because of this specific plant-environment relationship, vegetation on this kind of hill slope is much less dense than conventional soil-dominated hill slopes (Zhang, Hu, & Ni, 2013). Together with the physical barriers set by the rock matrix located between rock fractures (Hölscher, Hertel, Leuschner, & Hottkowitz, 2002; Schenk, 2006), root competition for water and other resources should be highly reduced. Moreover, it is theorized that the exploration of rock fractures by plant roots in this kind of environment is less about resource capture but more about space pre-emption (Carmo, Campos, & Jacobi, 2016;

Schwinning, 2010). That means that once a rock fracture is occupied by one plant, the exploration of this fracture by another plant becomes difficult which reduces competition. Furthermore, as described above, hill slopes were characterized by extremely high rock outcrop ratio thus water and nutrients can only be stored in randomly distributed rock fractures and crevices. Previous studies suggested that in this kind of stressful habitat, environmental filtering normally acted as strong selective forces during community assembly thus contributed to the assemble of species with similar environmental-adaptation strategies (Chapman & McEwan, 2018; Culmsee, &Leuschner, 2013). Accordingly, it is reasonable for co-existing plant species on rocky karst hill slopes to share similar main water sources and show inconspicuous hydrological niche segregation.

Although the dynamics of stem water isotope values of dominant species from one community converged to a similar pattern, specific differences in sources of water used by different species still exist. For example, our data from the SF provided clear evidence that *S. rotundifolium* (deciduous tree) utilized a certain percentage of water from a deep water pool (which was represented by phreatic groundwater) while *R. kwangsiensis* (evergreen shrub) could not be excluded from using water from the deep water pool; the relatively higher variation of stem water isotope values (than the tree) suggested that it utilized a smaller percentage of this stable water source and was more likely to rely on a mixture of rainwater received prior to each sampling time. From a different view, the tree and shrub from the SF exhibited smaller variations in stem water isotope values than the two species from the OS (Fig. 3). In accordance with these results, the overall variation in stem water isotope values in the SF was significantly smaller than that of the other two communities (Table 1). This fact in conjunction with the prediction that smoother dynamic of stem water isotope values throughout time is related to the utilization of rainwater received during a longer period, and the fact that phreatic groundwater represents a mixture of rainfall received within one year or more (Jones, Banner, & Humphrey, 2000), we can conclude that plants from the SF generally utilize far less fresh rainwater than plants from the other two communities. In other words, compared with plants from the SF, plants from the other two communities were more dependent on rainfall received within short time spans.

The primary reason for this discrepancy should be the contrasted species composition between communities. The OS and the DS were dominated by shrub and shrub-like deciduous tree species (Table S1), previous studies conducted in the same region revealed that these species normally employed shallow root systems and relied on water sources that derived from recent rainfall (Nie et al., 2011; Rong et al., 2011). Studies conducted in other karst regions (such as the Edwards Plateau, US) also provided evidence for similar water sources utilization by shrubland species (Elkington et al., 2014; Heilman et al., 2009). On the other hand, the SF was dominated by tree and big shrub species, studies have provided evidence that these kinds of species utilize water from deeper pools that could buffer the effects of rainfall received within short time spans (Gu et al., 2015; Nie et al., 2012). Since rainfall is highly erratic and uncertain, plants that rely on rainfall received within short time spans should frequently endure drought stress. Presumably, this is the reason that the same species in the OS were much smaller in size than that found in the SF (Table S1) and employed clear drought related leaf characteristics such as lower leaf water content and chlorophyll SPAD value (Fig. S2). In further studies, studying the response of water-related leaf physiological traits to rainfall pulse can generate more substantial evidence for the divergent reliance on rainfall received within short time spans.

Implications

Southwest China is one of the largest exposed carbonate bedrock areas (about 0.54 million km^2) in the world. Long-term climate data show that this region is experiencing a decreased number of rainy days, but increased heavy rain days and more extreme drought (Liu, Chen, Lian, Chen, & Chen, 2015); the vegetation recovery process may slow down or be reversed in unfavorable climate conditions (Jiang et al., 2014). Currently, most of the results associated with the response of plants to the variability of rainfall in karst regions are limited to a species level (except Pérez-Ramos et al., 2013), which results in knowledge gaps in understanding and forecasting the response of plant communities to projected rainfall changes (Clark et al., 2016). The current study provides a glimpse into plant water source discrepancies at a community level, which is further related to the divergent reliance on rainfall received within short time spans which could be helpful in evaluating the effects of rainfall variability on different communities. Although vegetation dynamics cannot be predicted solely on water availability for plants, our results indicate that early successional stage communities like the OS and the DS may be more vulnerable to rainfall concentration (decreased number of rainy days) at fine time scales than communities at late successional stages.

Conclusions

In this study, we demonstrate the existence of water source segregation among neighboring plant communities on rocky hill slopes that representing different vegetation successional stages in karst region of subtropical China. Contrary to the general idea of hydrological niche segregation among co-existing plant species, dominant species from the same community relied on a similar main water source and exhibited convergent dynamics of stem water isotope values over time. Dominant species of the secondary forest exhibited significantly smaller overall variation in stem water isotope values than that of the shrublands, which was agree with the fewer fluctuation of stem water isotope values of the common species in the secondary forest. As smoother dynamic of stem water isotope values is associated with the utilization of a mixture of rainwater received over a longer period of time, the secondary forest was estimated less dependent on rainwater received within short time spans than the shrublands. Our results provide substantial support for evaluating the responses of vegetation to the change of precipitation in subtropical karst region.

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Data accessibility: Primary data of this paper can be found through https://www.researchgate.net/profile/Yun-peng_Nie/contributions?ev=prf_act

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List of all appendices:

Table S1 Plot characteristics of three community type.

Table S2 Line-conditioned excess of plant stem water and rainfall.

Figure S1 Local Meteoric Water Line and isotope values of stem water.

Figure S2 Comparison of water-related leaf traits of the shared species.

Table 1 Overall variation in stem water δD values of species within community and the differences among communities (data were generated by treating each dominant species as a replicate).

	Open shrubland	Dense shrubland	Secondary forest
Average	-43.6±1.42a	-44.1±1.11a	-42.2±2.16a
Range	42.2±1.47b	52.8±2.95b	30.4±5.27a
SD	14.6±0.63b	17.7±1.29b	10.4±1.60a
CV	-0.31±0.022ab	-0.37±0.023b	-0.25±0.039a

Different letters in the same row represent significant difference among values at 0.05 level

Values are means ± SE.

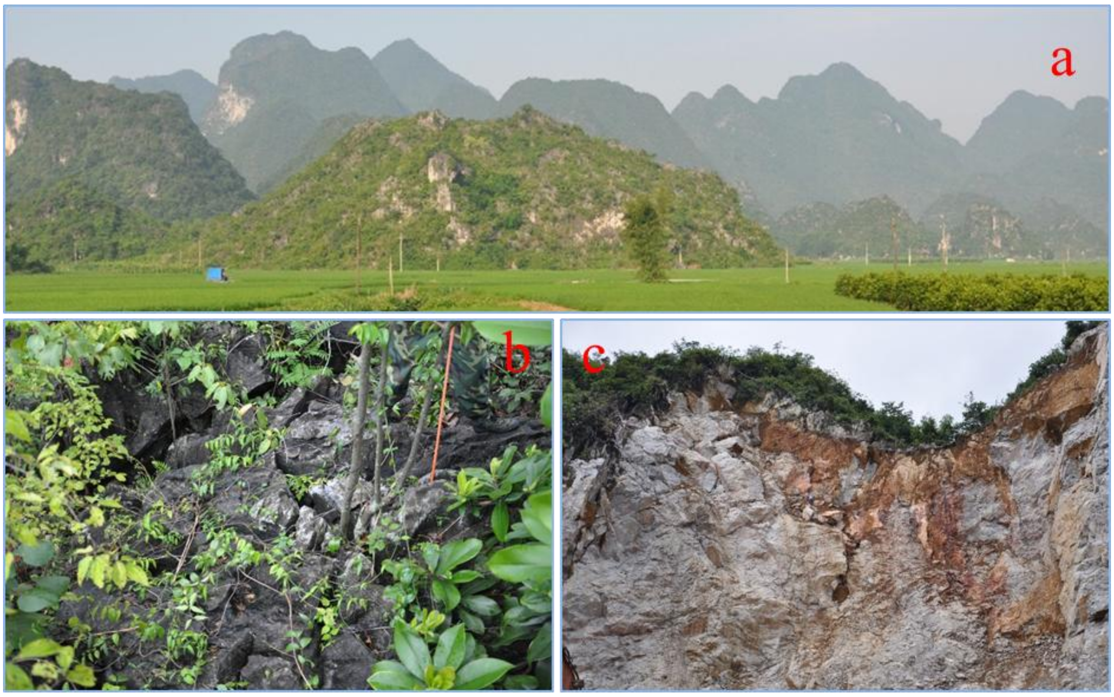

Fig. 1 The landscape (a) and rocky environment (b) of the watershed containing the sampling sites, as well as a big soil/bedrock profile (c) within the watershed.

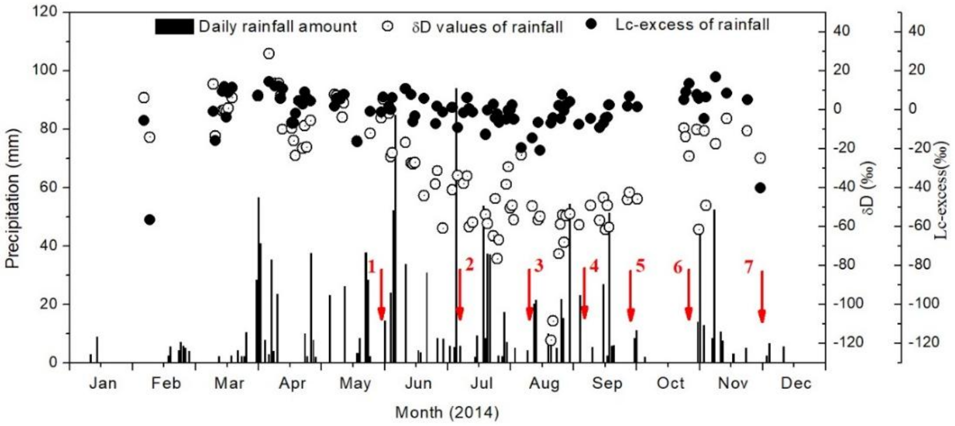

Fig. 2 Distribution of daily rainfall and fluctuation of rainwater δD values and line-conditioned excess (lc-excess) of rainfall throughout 2014. Numbered arrows indicate sampling times.

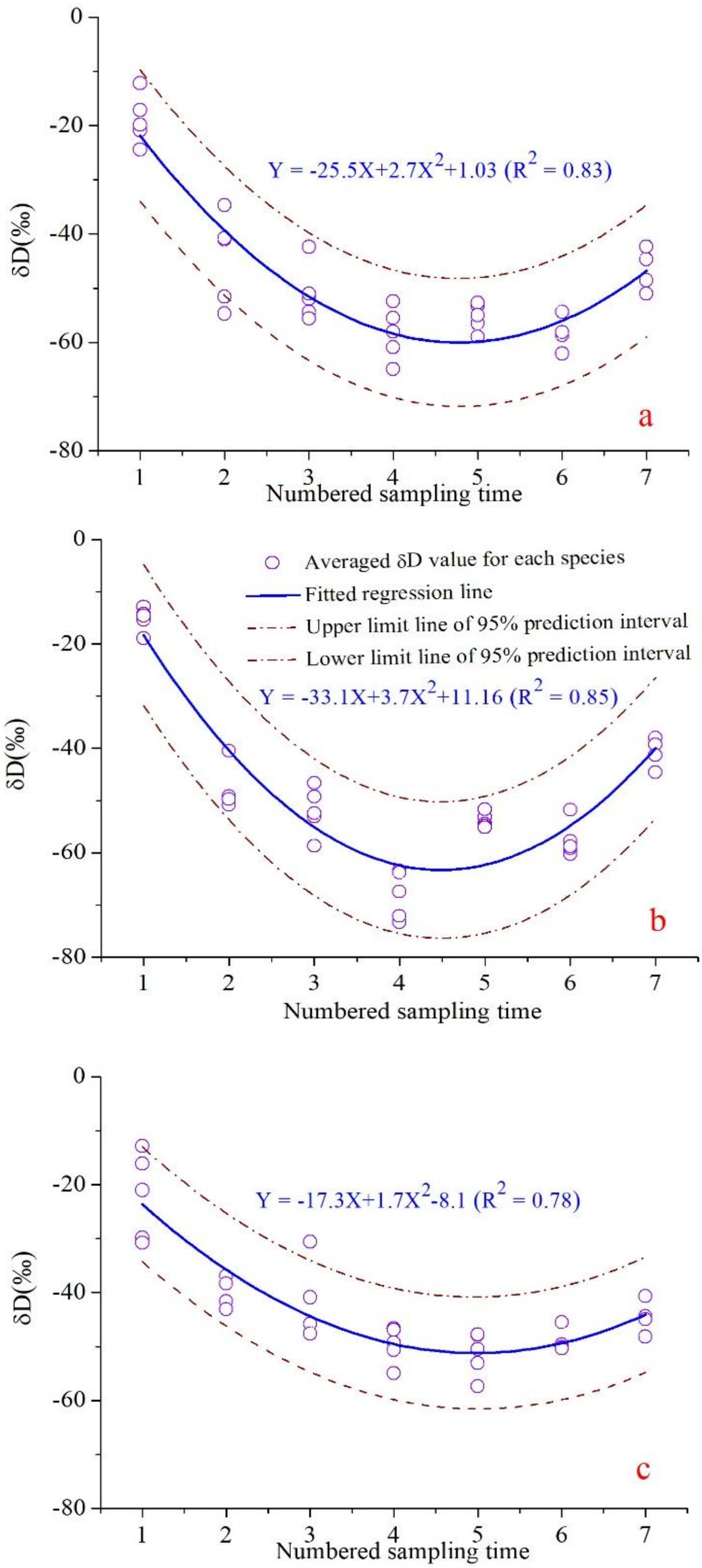
Y = -25.5X+2.7X^2+1.03 (R^2 = 0.83)
a
δD(‰)
0
-20
-40
-60
-80
1
2
3
4
5
6
7
Numbered sampling time
Averaged δD value for each species
Fitted regression line
Upper limit line of 95% prediction interval
Lower limit line of 95% prediction interval
Y = -33.1X+3.7X^2+11.16 (R^2 = 0.85)
b
Y = -17.3X+1.7X^2-8.1 (R^2 = 0.78)
c

Fig. 3 Integrated dynamic stem water isotope values of the dominant species within each community (a, open shrubland; b, dense shrubland; c, secondary forest). Each opened circle represents an averaged stem water isotope value of one species at a specific sampling time. Integrated dynamic of stem water isotope values of the dominant species within each community is represented by a solid regression line. Convergence of water source utilization by the dominant species from the same community was then identified based on most of the opened circles plotted between the upper and lower limit lines.

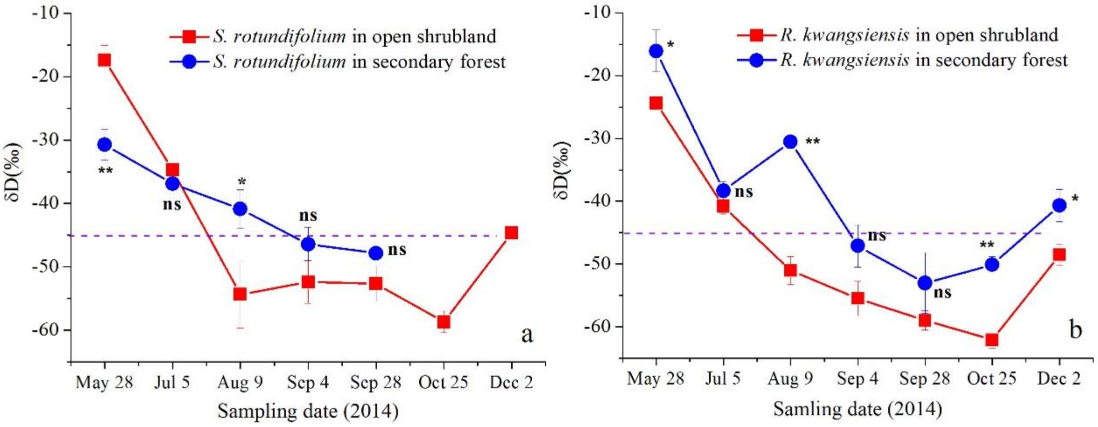

Fig. 4 Comparison of stem water isotope values of the shared species in the open shrubland and the secondary forest. The purple dashed lines indicate the relatively steady isotope values of phreatic groundwater. Significant differences between the same species from different community types at each sampling time are: $^{*}P < 0.05$; $^{**}P < 0.01$.