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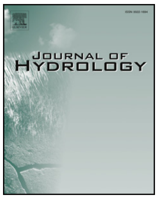

Research papers

Response of plants water uptake patterns to tunnels excavation based on stable isotopes in a karst trough valley

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ABSTRACT

Karstic aquifers are very sensitive and fragile to environmental changes. With more tunnels excavated in karst areas, groundwater levels are increasingly lowered and resulted in changed natural hydrogeological flow system and groundwater-dependent vegetation, soil and hydrology of surface water systems. Water, especially groundwater, is a limited and key factor and significant driving force for karst ecosystem processes. However, the effects of tunnels excavation on the ecosystems in karst areas remain largely unknown. This study aimed at investigating whether there were variations in soil water contents and shifts of plants water uptake patterns between rainy and dry seasons in a karst trough valley affected by tunnels excavation, and comparing their differences with those in a tunnel-free karst trough valley in southwest China. Monthly soil water contents at two soil layers of the upper 20 cm and lower 20–40 cm were measured, and soil water at two soil layers, subcutaneous water and plant (arbor and shrub) xylem water were sampled in February (mid dry season) and September (late rainy season) 2017, respectively, and the δ^2H and δ^{18}O of those waters were analyzed. The IsoSource model based on dual stable isotopes of δ^2H and δ^{18}O was used to estimate the contributions of different sources to the plant xylem water. Although the soil water contents at both valleys showed similar vertical and temporal variations resulted primarily from the evaporation and precipitation, the soil water contents of two soil layers in the tunneling affected valley were significantly lower than that of the tunnel-free valley at both seasons. Plants uptake water pattern changed from a dominant soil water source during the rainy season to a dominant subcutaneous water source during the dry season at both valleys. However, plants extracted more water from the subcutaneous zone in the tunneling affected karst trough valley than that of the tunnel-free karst trough valley at both seasons, of which the proportion of plants water uptake from the subcutaneous zone was 33% in rainy season and 76% in dry season, respectively, in the tunneling affected karst trough valley, while the proportion of plants water uptake from the subcutaneous zone was 24% in rainy season and 59% in dry season, respectively, in the tunnel-free karst trough valley. The above results indicated that tunnels excavation decreased the soil water contents and then changed plants water uptake patterns in karst areas.

1. Introduction

Nowadays, more and more tunnels are excavated due to more efficient transport critically dependent on highway and railway tunnel in the world. Up to the early 2010s, nearly ten thousand kilometers of railway tunnels alone have been built under a variety of complex geological conditions in China (Zhao et al., 2013). Generally, tunnels are excavated below groundwater table, especially in karst areas, and groundwater inflow into and then drainage out of the tunnels which result in a lowered groundwater table. Any groundwater drawdown could alter the natural hydrogeological flow system and consequently impact groundwater-dependent vegetation, soil and hydrology of surface water systems (springs, wells, streams, lakes, wetlands and the associated aquatic ecosystems). The more heterogeneous the physical environment is, the greater such risks. Karstic aquifers with double-layer hydrogeological structures are among the most problematical due to its sensitive and fragile to environmental changes (Ford and Williams, 2007). Therefore, most studies have mainly focused on the effects of tunnel excavation on karst hydrogeology (Gisbert et al., 2009; Vincenzi et al., 2009; Raposo et al., 2010; Chiocchini and Castaldi, 2011; Zarei et al., 2012; Alija et al., 2013; Zhao et al., 2013; Strozzi et al., 2014; Liu et al., 2015; Pujades et al., 2015).

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Water is a limited and key factor influencing plant growth in karst areas due to a very limited amount of water storage in the thin and rocky soils underlain by rock fractures. Such situation of water deficit for plant growth, resulting from groundwater drawdown and the drying up of surface water caused by tunnel excavation, could be more serious in karst areas. Although the effects of karst groundwater leakage and drawdown caused by tunnel excavation on vegetation are mentioned in previous studies (e.g. Vincenzi et al., 2009), no study appears to have been conducted on the effects of tunnel excavation-caused lowered groundwater on the karst ecosystems, such as the change of water sources for plant uptake or growth.

Stable isotopes of δ^2H and δ^{18}O in waters could provide an effective, powerful and nondestructive approach for identifying and partitioning the different potential water sources used by plants (White et al., 1985; Dawson and Ehleringer, 1991; Mccole and Stern, 2007; Querejeta et al., 2007; Rong et al., 2011; Ma and Song, 2016; Geris et al., 2017; Rothfuss and Javaux, 2017; Wang et al., 2017), because of no isotopic fractionation during terrestrial plant uptake of water (Wershaw et al., 1966; White et al., 1985; Dawson and Ehleringer, 1991; Dawson et al., 2002). Although all the water sources ultimately come from precipitation, values of δ^2H and δ^{18}O for various water sources are significantly different because of evapotranspiration. Thus, the hydrogen isotope content of xylem sap that does not expose it to evaporative enrichment reflects the water sources consumed by plants, and provides critical insights into adaptive responses of plants to the changing environments (Dawson et al., 2002).

In this study, we investigated soil moisture change of two soil layers of the upper 20 and lower 20–40 cm at two karst trough valleys with similar hydrogeological conditions but different impaction by tunnels excavation (affected/unaffected valley) in Chongqing of southwest China, and analyzed δ^2H and δ^{18}O values of the two dominant plant species (*Fraxinus chinensis Roxb*-arbor and *Viburnum chinshanense Graebn*-shrub) and three potential water sources available for plant uptake: rainwater captured by two soil layers of the upper 20 cm and lower 20–40 cm and the shallow groundwater (underlying subcutaneous water) in rainy and dry seasons at two karst trough valleys. We hypothesized that tunnels excavation lowered karst groundwater, and then could dry soil moisture and change the water sources for plant uptake during different seasons in karst areas. Thus, the new aspects addressed here are to quantify the proportional contribution of various water sources (soil water and subcutaneous water) to water sources of plant uptake in different seasons using the δ^2H and δ^{18}O of various waters impacted by tunnels excavation in karst trough valley areas.

2. Materials and methods

2.1. Study area

The Longfeng and Longche karst trough valleys (106°23′15″ ~ 106°28′05″E, 29°40′30″ ~ 29°48′10″N) with an area of 11.7 km^2 and 26.8 km^2, respectively, belong to the northern part of the Zhongliang Mountain located in the northwest of Chongqing, Southwest China (Fig. 1). The regional climate is characterized by a humid subtropical monsoon climate with the average annual precipitation of 1200 mm (over 80% of precipitation occurring during the rainy season from April to October) and the mean annual air temperature of 18 °C.

The Longfeng and Longche karst trough valleys are located in the Guanyinxia anticline, extending towards north/south, and consist of three ridges and two valleys (Fig. 1). The bedrock in the anticline core is the Lower Triassic Feixianguan Formation (T_1f) with an area of 9.2 km^2, which the first and third sections of the T_1f consist of gray limestone with a thickness of 120–170 m, and the second and fourth sections of the T_1f consist of purplish sandstone with a thickness of 310–380 m. On either side of the anticline core, there are Middle and Lower Triassic light grey to dark grey dolomite and limestone (T_2l and T_1j) with an area of 26.8 km^2 and a thickness of 500–700 m, which comprises the main karst aquifer, and Upper Triassic Xujiahe Formation (T_3xj) feldspar–quartz sandstone, shale and mudstone with an area of 2.5 km^2 and a thickness of 170–1100 m. Because the limestone and dolomite compared to the sandstone and shale can be easily corroded and eroded, karst trough valleys are developed in the T_1j and T_2l, while the ridges are formed in the erosion-resistant T_1f and T_3xj. In the karst trough valleys, the Triassic carbonate rock developed many vertical open fissures, providing basic conditions for forming karst depressions, fissures and sinkholes. Rainwater and surface water quickly drain through sinkholes, fissures or shaft into the karst aquifer and an underground river is developed in each karst trough valley.

The Feng and Long underground rivers are developed in the east and west of the Longfeng karst trough valley (Fig. 1), respectively, which the elevation of the outlet for the Feng and Long underground river is 302 m and 270 m, respectively. The length of the Feng and Long underground river is about 4.5 km and 5.2 km, respectively. Before 2000, the multi-annual mean discharge of the Feng and Long underground river was approximately 35 L/s and 25 L/s, respectively. However, from 2000 to 2017, the discharge of the Feng and Long underground river has gradually decreased to about 25 L/s and 15 L/s, respectively. The Qingfeng and Longche underground rivers are developed in the east and west of the Longche karst trough valley (Fig. 1), respectively, where the elevation of the outlet for the Qingfeng and Longche underground river is 560 m and 570 m, respectively. The length of the Qingfeng and Longche underground river is about 11.6 km and 11.8 km, respectively. The multi-annual mean discharge of the Qingfeng and Longche underground river is approximately 55 L/s and 45 L/s, respectively. Before 2000, there once were 15 epikarst springs which were sources of drinking and irrigation water, but only one epikarst spring with very low discharge can be found in the Longfeng karst trough valley in 2017. There are more than thirty wells and epikarst springs in the Longche karst trough valley.

Overlying soils are mainly the zonal yellow soil, of which the thickness is heterogeneous, varying from 10 cm to 100 cm. In the ridges of non-karst areas, the soil thickness is more than 50 cm, while the soil thickness is less than 50 cm in the karst area. The main land uses are forest land (21.6 km^2), agricultural land (13.3 km^2) and residential land (2.3 km^2) (Fig. 1). The evergreen wide-leaf forests (Fraxinus chinensis Roxb, Pagoda, etc.) and shrubs (Viburnum Chinshanense Graebn, etc.) are dominant vegetation. Most plants and shrubs are over 20-years due to the "Grain for Green" project which started in the middle of 1990s. However, the heights of arbors are usually low and most species fail to reach their biological heights due to limited available water stored in the karst thin soil.

2.2. Tunnels excavation in the Longfeng karst trough valley

The north–south lying Zhongliang Mountain, located in northwest of the Chongqing metropolitan area, is a big barrier to transportation. The construction of both railway and highway tunnels through the Zhongliang Mountain is necessary to improve the transportation. Three about 4-km-long tunnels without any ventilation shafts were built within an 8-km distance of the Longfeng karst trough valley during the past 20 years (Table 1), which were excavated below the water level of the underground rivers. The discharge of groundwater in three tunnels was high, but the inflow volume varied considerably (Table 1). Therefore, the groundwater level gradually lowered and surface water gradually dried up, resulting in the change of the paddy field to the dry land since the first tunnel excavation in the Longfeng karst trough valley (hereafter referred to as tunneling affected valley, abbreviated as "TAV"). Such situation was exacerbated after the second and third tunnel was constructed in the Longfeng karst trough valley. Now, 14 of 15 epikarst springs and most wells disappeared. Drinking water for human and livestock totally depends on the surface water pumped from the excavated tunnels and Jialing river, and most irrigation water is

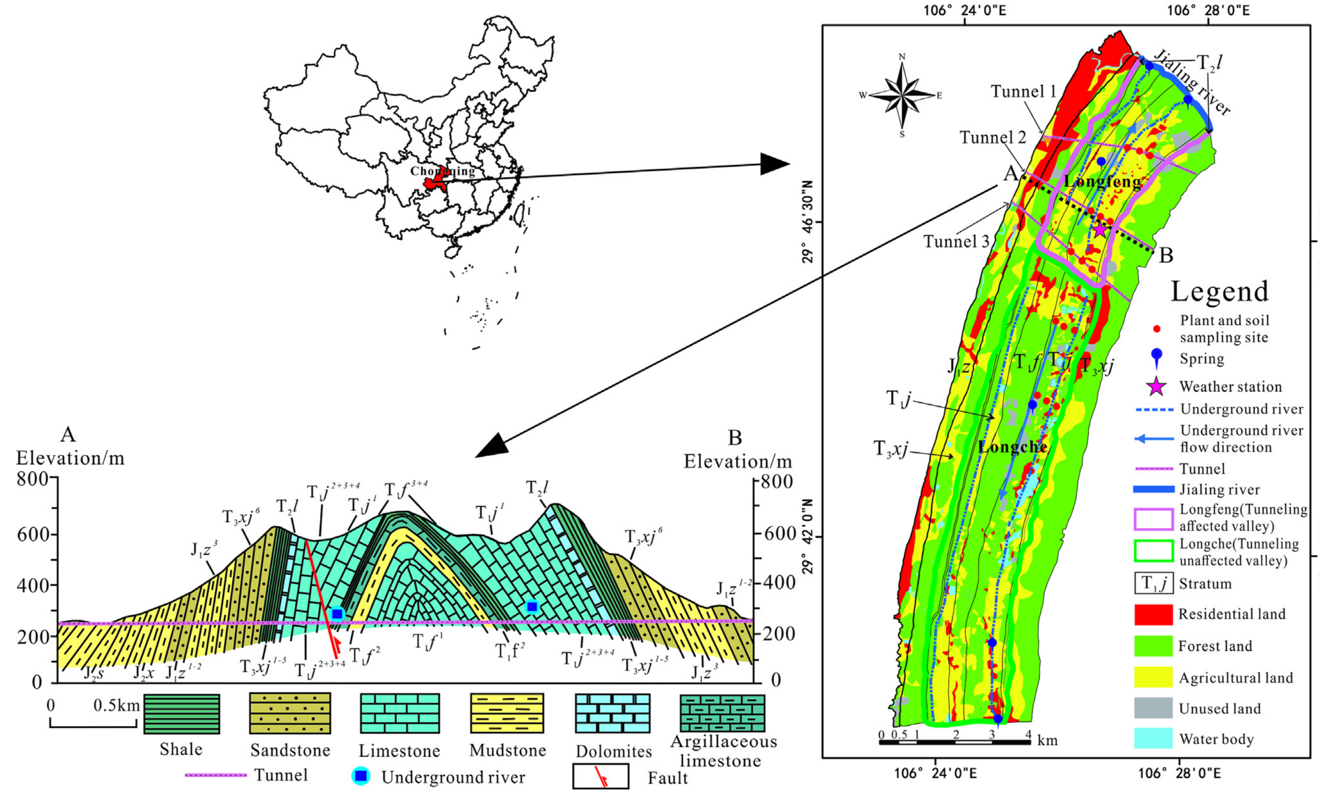

Fig. 1. Hydrogeology, land use, sampling points and geological cross-section of the Longfeng and Longche karst trough valleys in Chongqing.

from rainwater and part is from the surface water pumped from the excavated tunnels and Jialing river in the Longfeng karst trough valley. Meanwhile, the vegetation coverage becomes relatively poor and the rocky desertification worsens due to groundwater drawdown and the drying up of surface water caused by tunnel excavation in the Longfeng karst trough valley. As a control, tunnel excavation is absent in the Longche karst trough valley (hereafter referred to as tunneling unaffected valley, abbreviated as “TUAV”), and it is a natural karst hydrogeological system. Thus, this provides a unique opportunity to study the impacts of tunnels excavation on the hydrogeology, hydrology and eco-environment in karst areas.

2.3. Sampling

The air temperature and precipitation, recorded at 15-min intervals with a resolution of 0.01 °C and 0.1 mm, respectively, by the HOBO field weather stations located in the Longfeng karst trough valley (Fig. 1), were collected from January to December 2017.

Soil samples at the depth of 0–20 and 20–40 cm for analysis of soil water contents were collected monthly in 2017. Samples of plant xylem water, soil water at the depth of 0–20 and 20–40 cm and spring water (subcutaneous water) for isotopic analysis (δ^2H and $\delta^{18}O$) were collected in the dry season of February 2017 and in the rainy season of September 2017.

Sampling of soils and plant species was conducted from 5 cross-sections of the karst trough valleys, of which 3 cross-sections were located at the top of three tunnels of the Longfeng karst trough valley, and 2 cross-sections were located in the Longche karst trough valley (Fig. 1). There are 3 sampling points at each cross-section, of which 2 sampling points were located on both sides of the hillside and 1 was located in the bottom of valley. At each sampling point, soils and plant species were sampled if both arbors and shrubs could be found, respectively.

Fraxinus chinensis Roxb and *Viburnum chinshanense* Graebn are typical aboriginal species of arbors and shrubs, and were sampled at each sampling point. For each plant species, three to five individuals with similar diameter of breast height were selected. All the plant samples were cut into 3–4 cm segments, immediately placed into glass vials with screw caps, sealed with polyethylene parafilm and kept frozen in a freezer (−20 °C) for isotopic analysis. The total samples during every measurement period were 7 and 3 for the species of arbor and shrub at the tunneling affected valley, and 5 and 3 for the species of arbor and shrub at the tunnel-free valley, respectively.

At each sampling point, two soil samples were collected using the hand probes at the depths of 0–20 and 20–40 cm below ground. A total of 360 soil samples collected monthly were used to obtain gravimetric soil moisture content (SMC, %) as determined by drying at 105 °C for 24 h. The total soil samples for isotopic analysis during every measurement period were 10 and 8 in the tunneling affected and the tunnel-free valley at rainy and dry season, respectively.

Meanwhile, a total of 6 subcutaneous waters, 1 epikarst spring and 2 outlets of underground river at the tunneling affected valley, and 2

Table 1
Basic information of three tunnels in the Longfeng karst trough valley.

Tunnel No.	Tunnel name	Excavation period	Tunnel length (m)	East/west elevation (m)	East/west discharge (L/s)
1	Shijialiang tunnel of Chongqing ring expressway	2006–2008	4285	260/245	2.3/6.5
2	Beibei tunnel of Yuwu expressway	1999–2001	4035	250/240	2.5/16.8
3	Beibei tunnel of the 6th metro Line	2010–2013	4322	245/240	1.5/23.3

epikarst springs and 1 outlets of underground river at the tunnel-free valley, were collected at rainy and dry season to analyze isotopic compositions (δ^2H and δ^{18}O). Mineral oil was applied to each bottle to prevent evaporation.

2.4. Isotopic analyses

Water in plant and soil samples was extracted using a cryogenic vacuum distillation system (LI-2100, LICA, Beijing, China). The extraction efficiency was over 99%, which was sufficient to obtain unfractionated water samples. The extracted water sample is loaded into a 2 ml brown bottle and sealed with a sealing membrane and stored in a refrigerator at 4 °C until subsequent isotopic analysis.

The δ^2H and δ^{18}O values of subcutaneous water and water in plant tissues and soils were measured with an Isotopic Ratio Infrared Spectroscopy (IRIS) system (DLT-100; Los Gatos Research, Mountain View, USA) at Chongqing Key Laboratory of Karst Environment, Southwest University. The isotopic ratios of δ^2H-H_2O and δ^{18}O-H_2O were expressed as a permil (‰) deviation from Vienna Standard Mean Ocean Water (VSMOW). The analytical precision of the liquid water measured by IRIS was ± 1.2‰ for δ^2H and ± 0.3‰ for δ^{18}O.

2.5. Data analysis

The isotopic compositions of all potential water sources and xylem water were entered into the IsoSource model (Phillips and Gregg, 2003) to calculate the contribution of each source to stem water. IsoSource is a Microsoft Visual Basic™ software package which calculates ranges of source proportional contributions to a mixture based on stable isotope analyses when the number of sources is too large to permit a unique solution and can provide the distribution of source proportions which are consistent with isotopic mass balance (Phillips and Gregg, 2003). The raw xylem isotope values of δ^2H and δ^{18}O in two plant species were used as mixture data inputs into IsoSource model, and isotope values of δ^2H and δ^{18}O from various soils horizons (0–20 and 20–40 cm) and subcutaneous water were used as source data and input into IsoSource model. To guarantee that no legitimate feasible source combinations was missed, the fractional increment employed in our calculations was 0.01, 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 0.01‰ in our calculations.

Statistical analysis was conducted using the SPSS 20.0 program (SPSS Inc., Chicago, IL, USA). Paired-sample *t* test was used to detect significant differences in the soil moisture contents and isotopic compositions of soil and xylem water between the tunneling affected and tunnel-free karst trough valleys during dry and rainy seasons.

3. Results

3.1. Seasonal variations of precipitation and soil moisture contents

The total precipitation was 1314 mm in 2017 and 86.5% occurred during the vegetation growing season (from April to October), accompanying with a severe summer drought in August. The highest monthly precipitation was 249 mm in September, accounting for 19% of the year's total precipitation, while the lowest monthly precipitation was only 10 mm in December (Fig. 2).

As shown in Fig. 2, marked vertical and seasonal variations in soil moisture contents were found at both valleys. The soil moisture contents increased with the increase of soil depths at both seasons, which the average of soil moisture contents in upper and lower soil layers was 24% and 27%, respectively. Meanwhile, the soil moisture contents of different soil depths at two valleys exhibited significant temporal changes with the precipitation variations. The highest soil moisture contents occurred in June (about 32%), while lowest soil moisture contents (about 20%) occurred in August with highest temperature and summer drought.

Meanwhile, soil moisture contents showed significant difference in upper soil layers ($P < 0.001$) and lower soil layers ($P < 0.001$) between two karst valleys (Fig. 2), although the soil moisture contents of two valleys showed similar vertical and temporal variations at both seasons. The soil moisture contents for the depths of 0–20 cm and 20–40 cm varied from 17% to 28% with a mean of 22.3% and 19% to 32% with a mean of 25.3% in the tunneling affected valley, and 20% to 32% with a mean of 25.9% and 24% to 38% with a mean of 29.4% in the tunnel-free valley, respectively. Such obvious difference in soil moisture contents between two valleys, showing lower soil moisture contents of both soil layers in the tunneling affected valley, indicated that tunnels excavation could dry soil moisture contents in karst areas.

3.2. Variations in isotopic compositions of soil water, subcutaneous water and plant xylem water

3.2.1. Variations in isotopic compositions of soil water in the tunneling affected and tunneling unaffected karst trough valley

The isotopic compositions of the soil water changed with the seasons and soil depths at both valleys (Fig. 3 and Table 2). The δ^2H and δ^{18}O values of soil water were less negative in the dry season than those in the rainy season at both valleys. Meanwhile, the isotopic compositions of the soil water showed significant difference between two valleys at both seasons ($P < 0.001$ in dry season, and $P < 0.05$ in rainy season, respectively), which the isotopic compositions of the soil water was less negative in the tunneling affected valley than those in the tunnel-free valley at both seasons. In the dry season, the mean values of δ^2H and δ^{18}O in soil water were −58.89‰ and −8.55‰ in the tunnel-free valley, and −54.77‰ and −7.46‰ in the tunneling affected valley, respectively. In the rainy season, the mean values of δ^2H and δ^{18}O in soil water were −96.61‰ and −16.40‰ in the tunnel-free valley, and −91.10‰ and −14.83‰ in the tunneling affected valley, respectively.

3.2.2. Variations in isotopic compositions of subcutaneous water in the tunneling affected and tunneling unaffected karst trough valley

Similarly, the δ^2H and δ^{18}O values of subcutaneous water showed slighter depleted in the rainy season than those in the dry season at both valleys. However, compared to the seasonal variations of the δ^2H and δ^{18}O from the soils water, less variation of those values from the subcutaneous water were observed in both valleys at both seasons (Fig. 3 and Table 2).

3.2.3. Variations in isotopic compositions of plant xylem water in the tunneling affected and tunneling unaffected karst trough valley

As shown in Fig. 3 and Table 2, there were negligible differences in the δ^2H and δ^{18}O values between the stem xylem water of arbors and shrubs, implying that the arbors and shrubs absorbed water from the similar water sources in the karst areas. However, marked seasonal differences in the δ^2H and δ^{18}O values of stem xylem water were observed at both valleys, suggesting that plant water uptake had significant temporal variability.

Furthermore, the isotopic compositions of the stem xylem water showed significant difference between two valleys at both seasons ($P < 0.001$ at both seasons), showing less negative values in the tunneling affected valley than those in the tunnel-free valley. In the dry season, the mean values of δ^2H and δ^{18}O in stem xylem water were −47.72‰ and −6.98‰ in the tunneling affected valley, and −49.40‰ and −7.44‰ in the tunnel-free valley, repectively. In the rainy season, the mean values of δ^2H and δ^{18}O in stem xylem water were −78.94‰ and −12.85‰ in the tunneling affected valley, and −86.39‰ and −14.31‰ in the tunnel-free valley, respectively. Such differences in the δ^2H and δ^{18}O values of stem xylem water between two valleys at both seasons indicated that the water uptake patterns of

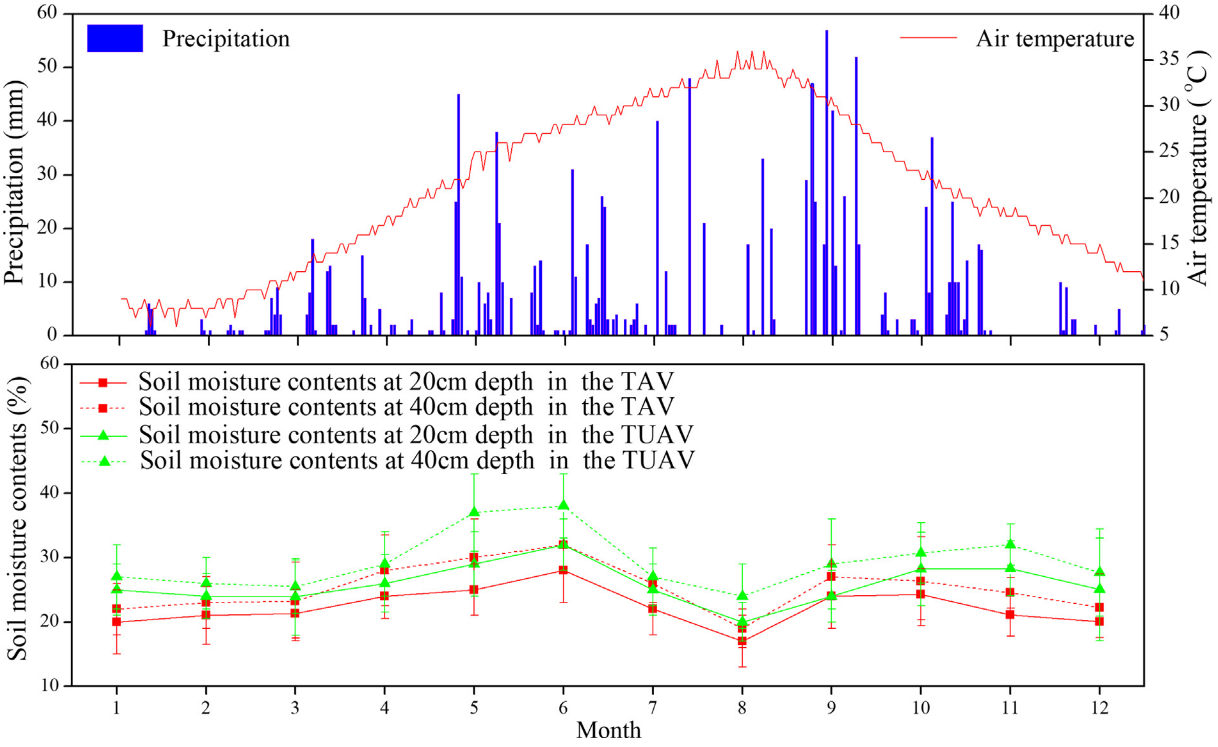

Fig. 2. Variations of air temperature, precipitation and soil moisture contents.

plants in the tunneling affected valley could be changed by the tunnels excavation.

3.2.4. *IsoSource estimation of feasible contributions of potential water sources to plant water uptake at two valleys*

As indicated by IsoSource model, there were marked seasonal variations in the proportions of the different water sources for plant uptake at both valleys (Table 3). During the rainy season, plants mostly utilized water from the soil layers, and then from the subcutaneous zone at both karst trough valleys. Contrasted, during the dry season, plants predominantly extracted water from the subcutaneous zone, and then from the soil layers at both karst trough valleys.

Furthermore, the IsoSource model revealed that there were significant differences in plants water uptake patterns between the tunneling affected valley and tunnel-free valley, which plants extracted obviously more water from the subcutaneous zone at the tunneling affected karst trough valley than that at the tunnel-free karst trough valley at both seasons (Table 3 and Fig. 5). During the rainy season, the

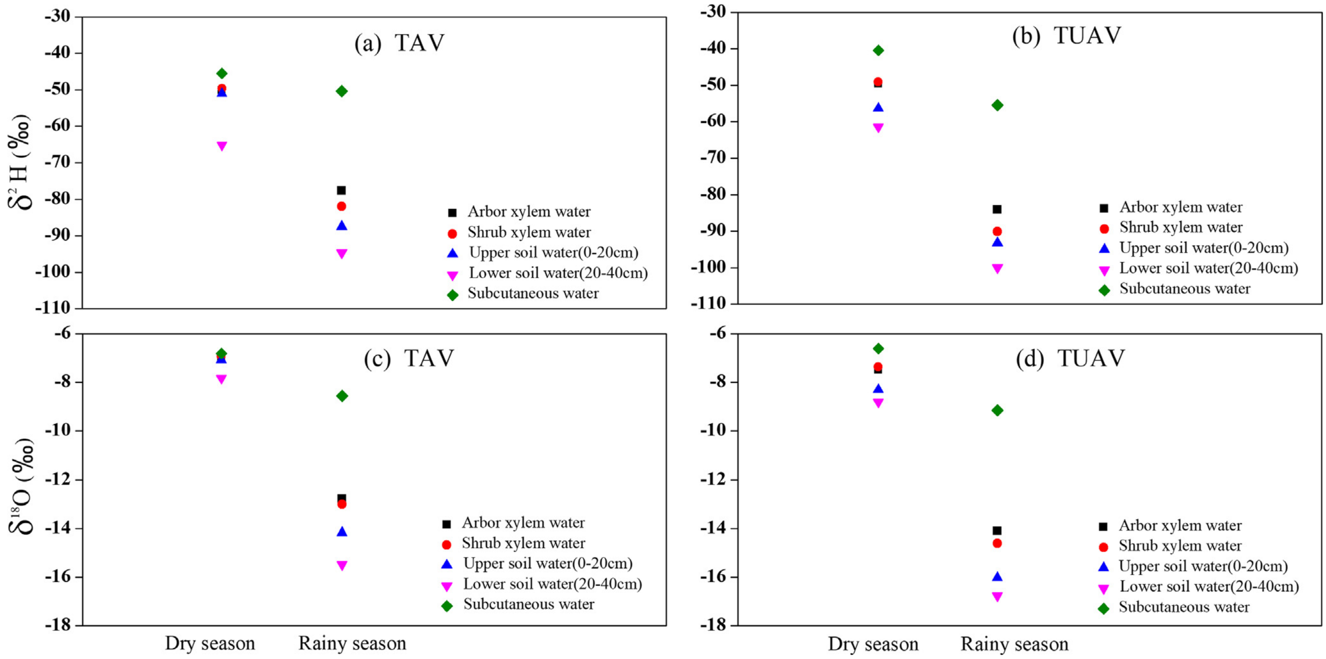

Fig. 3. Seasonal variations of δ^2H and δ^{18}O in waters of plant xylem, upper soil, lower soil and subcutaneous zone in tunneling affected/unaffected karst trough valley.

Table 2
Seasonal variations in δ^2H and δ^{18}O of plant xylem water, soil water and subcutaneous water in the Longfeng and Longche karst trough valleys.

Valley	Vegetation types	Water sources		Dry season		Rainy season	
				δ^2H (‰)	δ^{18}O(‰)	δ^2H (‰)	δ^{18}O(‰)
Longfeng (Tunneling affected karst trough valley)	Fraxinus chinensis Roxb	Arbor (n = 7)	min	−49.10	−7.15	−83.01	−13.24
			max	−46.78	−6.78	−74.96	−12.45
			mean	**−47.85**	**−7.00**	**−77.64**	**−12.78**
	Viburnum chinshanense Graebn	Shrub (n = 3)	min	−47.86	−7.01	−84.39	−13.83
			max	−46.70	−6.81	−79.45	−12.35
			mean	**−47.40**	**−6.93**	**−81.98**	**−13.00**
	Upper soil water (n = 10)		min	−52.99	−7.31	−89.04	−15.13
			max	−46.64	−6.94	−85.77	−13.04
			mean	**−48.62**	**−7.08**	**−87.55**	**−14.18**
	Lower soil water (n = 10)		min	−62.73	−7.96	−108.14	−17.43
			max	−60.12	−7.58	−89.74	−14.28
			mean	**−60.93**	**−7.83**	**−94.66**	**−15.48**
	Subcutaneous water (n = 3)		min	−45.90	−7.12	−51.26	−8.94
			max	−41.51	−6.57	−49.61	−8.27
			mean	**−43.76**	**−6.81**	**−50.30**	**−8.55**
Longche (Tunneling unaffected karst trough valley)	Fraxinus chinensis Roxb	Arbor (n = 5)	min	−50.87	−7.63	−95.41	−15.44
			max	−47.85	−7.29	−78.17	−12.97
			mean	**−49.59**	**−7.48**	**−84.14**	**−14.12**
	Viburnum chinshanense Graebn	Shrub (n = 3)	min	−49.45	−7.50	−96.06	−14.79
			max	−48.79	−7.15	−82.12	−14.50
			mean	**−49.09**	**−7.37**	**−90.14**	**−14.62**
	Upper soil water (n = 8)		min	−58.15	−8.52	−104.38	−16.81
			max	−54.76	−8.05	−82.92	−13.54
			mean	**−56.33**	**−8.30**	**−93.25**	**−16.04**
	Lower soil water (n = 8)		min	−65.48	−8.93	−110.66	−17.82
			max	−58.31	−8.76	−89.72	−14.74
			mean	**−61.44**	**−8.80**	**−99.97**	**−16.77**
	Subcutaneous water (n = 3)		min	−42.64	−6.87	−57.54	−9.64
			max	−38.90	−6.40	−53.54	−8.87
			mean	**−40.41**	**−6.60**	**−55.40**	**−9.15**

proportions of plants water uptake from the upper and lower soil layers and the subcutaneous zone were 29% (varying from 23% to 36%), 47% (varying from 37% to 57%) and 24% (varying from 13% to 37%) respectively in the tunnel-free karst trough valley, while the proportions of plants water uptake from the upper and lower soil layers and the subcutaneous zone were 23% (varying from 11% to 34%), 44% (varying from 29% to 71%) and 33% (varying from 18% to 50%) respectively in the tunneling affected karst trough valley. During the dry season, the proportions of plants water uptake from the upper and lower soil layers and the subcutaneous zone were 17% (varying from 13% to 20%), 24% (varying from 17% to 31%) and 59% (varying from 53% to 68%) respectively in the tunnel-free karst trough valley, while the proportions of plants water uptake from the upper and lower soil layers and the subcutaneous zone were 10% (varying from 8% to 12%), 14% (varying from 10% to 20%) and 76% (varying from 70% to 82%), respectively, in the tunneling affected karst trough valley. This indicated that the plants water uptake patterns have been changed by the tunnels excavation in the tunneling affected karst trough valley.

4. Discussion

4.1. Differences in seasonal soil moisture contents between two karst trough valleys: precipitation vs. tunnels excavation

The soil water contents at both valleys showed higher values during the rainy season due to plenty rainfall, while soil water contents at both valleys showed lower values during the dry season because of less precipitation. Thus, more than 10% of the seasonal variations in soil water contents at both valleys are caused by the seasonal changes of precipitation.

Compared to the tunnel-free karst trough valley, the lowered soil moisture contents of different soil layers in the tunneling affected karst trough valley at both seasons might result from the tunnels excavation. The modification of natural hydrogeological flow system caused by the tunnels excavation, especially drilled below underground river, could accelerate groundwater flow velocities (Vincenzi et al., 2009), and consequently result in a rapid and great loss of soil and soil water along with the cracks and crevices developed in limestone, especially in the

Table 3
Proportions of feasible water sources (%) for the species in the Longfeng and Longche kart valleys at the mid dry season and late rainy season in 2017. Average source proportions calculated by the IsoSource model are shown, as well as the range of minimum/maximum source proportions (in parentheses).

Valley	Water sources	Arbors		Shrubs		All species	
		Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season
Longfeng (Tunneling affected karst Trough valley)	Upper soil water	10(8–12)	22(11–28)	11(9–11)	28(19–34)	10(8–12)	23(11–34)
	Lower soil water	14(10–20)	41(29–71)	15(12–20)	49(43–61)	15(10–20)	44(29–71)
	Subcutaneous water	76(70–82)	37(18–50)	74(71–77)	23(20–27)	75(70–82)	33(18–50)
Longche (Tunneling unaffected karst Trough valley)	Upper soil water	16(13–18)	28(23–36)	18(17–20)	30(29–30)	17(13–20)	29(23–36)
	Lower soil water	23(17–31)	44(37–54)	25(24–26)	53(49–57)	24(17–31)	47(37–57)
	Subcutaneous water	61(53–68)	28(15–37)	57(56–57)	17(13–21)	59(53–68)	24(13–37)

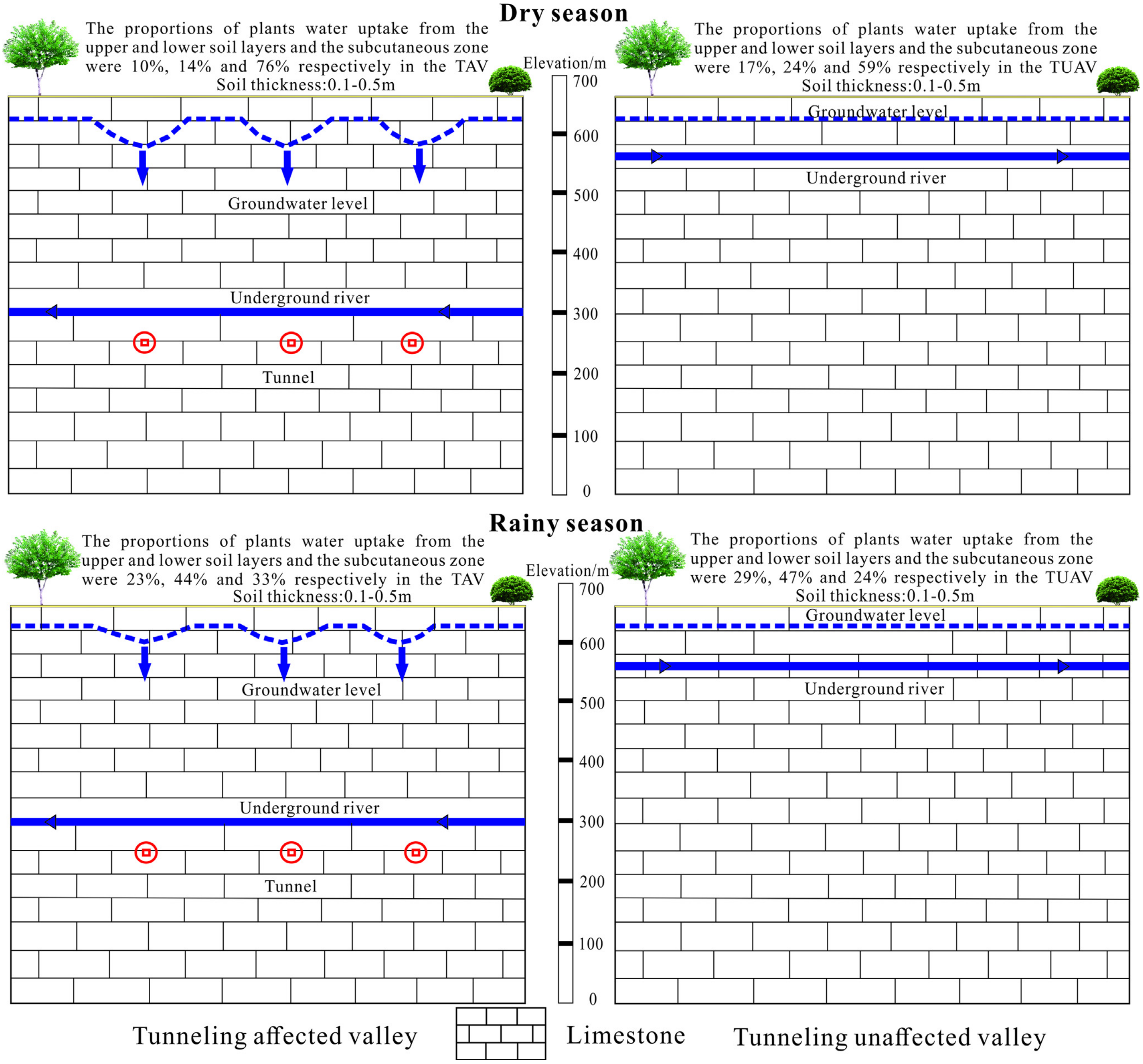

Fig. 4. Conceptual model of tunnels excavation impacts on plants water uptake patterns in karst trough valley.

rainy season. As shown in Fig. 2, the mean values of soil water contents at the upper and lower soil layers in the tunneling affected karst trough valley at rainy and dry season were about 3% and 4%, and 5% and 5% lower than those in the tunnel-free karst trough valley, respectively. This supports our hypothesis that tunnels excavation could lower karst groundwater and then dry soil moisture contents.

4.2. Differences in seasonal water uptake patterns of plants between two karst trough valleys: soil water vs. subcutaneous water, and impact from tunnels excavation

Marked seasonal variations in water uptake patterns of plants (arbors and shrubs) at the both valleys are attributed to the dimorphic root system of plants growing in subtropics karst areas: a network of branched surface roots potentially takes up water from upper soil layers, while deeper roots extract water stored in epikarst and even underground river that has been recharged from antecedent precipitation (Williams and Ehleringer, 2000; Kulmatiski et al., 2006; Heilman et al., 2009; Hasselquist et al., 2010; Nie et al., 2010). Such dimorphic root system seems to be more important for species growing on karst shallow soils because these shallow soils are deficient of water to support plant growth (Hasselquist et al., 2010) and plants have to extract water from the subcutaneous zone or even from underground river. As indicated by some studies, although shallow bedrock may hamper the downward growth of roots in karst thin soils, weathered limestone bedrock often allows roots penetration through a network of cracks, fissures and channels to extract waters from the soil layers and subcutaneous zone, and even from deep underground river (White et al., 1985; Canadell et al., 1996; Jackson et al., 1999; Mccole and Stern, 2007; Querejeta et al., 2007; Hasselquist et al., 2010; Nie et al., 2010; Estrada-Medina et al., 2013; Heilman et al., 2009, 2014). Jackson et al. (1999) revealed that some plants in karst areas can grow their roots deeper than 65 m to use the deep groundwater. The selected plants, *Fraxinus chinensis* Roxb and *Viburnum chinshanense* Graebn, have developed dimorphic root systems (Fig. 5). Thus, plants in karst areas could derive their most water from soil layers through the surface roots during the rainy season due to lots of precipitation resulted in ample soil water at upper and lower soil layers, while plants have to use the deep roots to extract

Fig. 5. Photograph of *Fraxinus chinensis Roxb* showing dimorphic root system.

primarily water from the subcutaneous zone during the dry season due to the decrease in the soil water at upper and lower soil layers with the decline of precipitation.

However, it should be noted that the subcutaneous water contributed a lot to plant water sources at both valleys (about 24% at the tunnel-free karst trough valley and 33% at the tunneling affect karst trough valley, respectively) even in rainy season (Fig. 4), although the soil layers, especially the lower soil layer, could provide much water for plants utilization. This is resulted from the low rainwater holding capacity in karst discontinuous shallow soil layers (Hasselquist et al., 2010; Nie et al., 2010; Rong et al., 2011) and the gradual increase in transpiration of plants during the continuous growing season. Similar results were identified in other karst areas (Nie et al., 2010; Rong et al., 2011) in which both evergreen arbors and shrubs used a mixture water from the soil layers and subcutaneous zone during the rainy season.

Furthermore, plants extracted more water from the subcutaneous zone (about 9% at rainy season and 17% at dry season, respectively) at the tunneling affected karst trough valley than that at the tunnel-free karst trough valley at both seasons (Fig. 5), because there are marked lower soil water contents at upper and lower soil layers in the tunneling affected karst trough valley than that in the tunnel-free karst trough valley at both seasons (Fig. 2). Evergreen species in karst areas can maintain their foliage and growth even in the drought season, suggesting they have access to a more permanent water source, such as groundwater (White et al., 1985). Although soil water contents decreased and groundwater lowered caused by the tunnels excavation, plants could extract water from the deep zone for growth through the deep roots in the tunneling affected karst trough valley.

More water of plants uptake extracted from the subcutaneous zone at both seasons should be attributed to the decrease of the soil water availability caused by tunnels excavation in the tunneling affected karst trough valley. Native plants in karst areas are much more flexible, resulting from the dimorphic root system, in allocating roots and adjusting water uptake in the soil layers and subcutaneous zone with high water resources availability. Such flexibility can help plants in karst areas to capture belowground water with fluctuated depth through time, because the availability of water in soil layers and subcutaneous zone may vary with the seasonal pattern of precipitation in the monsoon climate areas, and causing by tunnels excavation in the karst areas.

5. Conclusions

Soil water contents and the stable isotopes of δ^2H and δ^{18}O were used to detect whether there were seasonal variations in soil water contents and shifts of plants water uptake patterns between rainy and dry seasons in two karst trough valleys with similar hydrogeological conditions but different impaction by tunnels excavation (affected/unaffected valley) of southwest China. Marked vertical and seasonal differences in the soil water contents were found between two valleys, but the soil water contents of different soil layers at both seasons in the tunneling affected valley were always lower than those in the tunnel-free valley. Also, there were significant seasonal differences in the δ^2H and δ^{18}O values of stem xylem water in both valleys, showing less negative isotopic values in the tunneling affected valley than those in the tunnel-free valley at both seasons. The IsoSource outcomes indicated that plants uptake water pattern changed from a dominant soil water source during the rainy season to a dominant subcutaneous water source during the dry season at both valleys, showing a greater degree of ecological plasticity of plants in karst areas. This is mainly attributed to the functionally dimorphic root system of native plants in karst areas. However, plants extracted obviously more water from the subcutaneous zone in the tunneling affected karst trough valley than that in the tunneling unaffected karst trough valley at both seasons. Decreasing soil water availability resulted from tunnels excavation contributes to this variation. The flexible water uptake patterns of plants might help plants to capture belowground water and nutritional sources, and adapt to the fluctuating environment in karst areas. This study provides a useful method to explore the effects of tunnels excavation on karst water resources and ecosystem, and to inform better measures to protect karst region eco-environments.

Conflict of interest

The authors declare that there are no conflicts of interest.

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