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Increasing leaf δ^{13}C values of woody plants in response to water stress induced by tunnel excavation in a karst trough valley: Implication for improving water-use efficiency

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ABSTRACT

Plant species growing in karst shallow-soil areas always experience water deficit especially in seasonally dry. Moreover, such water stress is aggravated by tunnel excavation in karst areas. However, the effects of tunnel excavation on karst ecosystems remain largely unknown. This study aimed at investigating whether there were variations in soil water contents and shifts of water use strategies of plants between rainy and dry seasons in a karst trough valley affected by tunnel 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 0–20 cm and the lower 20–40 cm were measured from January 2017 to December 2018. Foliage of woody plants were sampled in the dry season of December 2017 and March 2018, and in the rainy season of June and September 2018, respectively, and leaf δ^{13}C values were analyzed. The soil water contents at both valleys showed significant seasonal variations, and 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. Plant water use strategy changed from profligate water-use pattern in the rainy season to conservative water-use pattern in the dry season. Moreover, increased leaf δ^{13}C values at both seasons in the tunneling affected valley suggests that the water stress resulted from the tunnel excavation has significantly impacted the physiological process of plants in karst areas. These results highlight that improving water use efficiency (WUE) is a common water use strategy to overcome water limitation for native plants in karst areas during unfavorable drought conditions.

1. Introduction

Karst, a special landscape shaped by the dissolution of carbonates, covers about 20% of the Earth's dry ice-free land, and karst aquifers are at least a partial source of drinking water supply to almost a quarter of the world's population (Ford and Williams, 2007). However, carbonates often occupy landscapes where water shortages are common due to pronounced seasonality in rainfall and double-layer hydrogeological structures resulting in a very limited amount of water storage capacity with significant seasonal variation in the thin and rocky karst soils (Ford and Williams, 2007). Therefore, drought (water) stress is one of the most important factors in limiting the photosynthesis, growth, and survival of plants in karst habitats (White et al., 1985; Jackson et al., 1999; Mccole and Stern, 2007; Querejeta et al., 2007; Hasselquist et al., 2010; Heilman et al., 2009, 2014; Nie et al., 2010, 2014). Furthermore, such stress on karst water resources resulted from the climate change (increasing temperature and precipitation) and excessive groundwater extraction for agriculture, industry and urban has increased significantly in recent decades (Gleeson et al., 2012; Taylor et al., 2012; Hartmann et al., 2014), because karst is particularly vulnerable to environment changes and human impacts due to their unique hydrogeological characteristics (Ford and Williams, 2007). Meanwhile, plateau and mountains are the dominant landforms in karst areas, especially in Europe and Asia, resulting in the insurmountable barriers to transport. As a result, excavation of tunnels has been a common necessity for efficient transport of both motorways and railways in these areas (Gisbert et al., 2009; Vincenzi et al., 2009; Butscher et al., 2011; Zarei et al., 2012; Liu et al., 2019). Generally, tunnels are excavated below groundwater table in karst areas, although some tunnels in karst mountains may start in the vadose zone at each end but pass into a transient zone or even a steady-state phreatic zone in their central parts, creating an elongated zone of depression to permit it to drain gravitationally, resulting in a lowered groundwater table (Ford and Williams, 2007; Vincenzi et al., 2009). Karst groundwater drawdown

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can lead to drying up of karst spring discharges (Gisbert et al., 2009; Liu et al., 2019), and losing or drying up of surface water completely (Vincenzi et al., 2009; Liu et al., 2019), and then decreasing soil water contents and plants extracting more groundwater (Liu et al., 2019). Therefore, 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) and associated ecosystems in karst areas. Furthermore, such karst water stress for plant growth is likely to increase dramatically in the future, resulted from an increasing tunnel excavation-induced groundwater drawdown with fast development of transportation and urbanization in karst areas, especially in Asia, such as China.

Although karst shallow-soil with a limited water supply is a big challenge for plant growth and survival, lots of plants, including broad-leaved evergreen species, can grow and survive in such water-limited environments, suggesting those plant species have developed various mechanisms for drought adaptation, including development of deep roots (White et al., 1985; Canadell et al., 1996; Jackson et al., 1999; Mccole and Stern, 2007; Querejeta et al., 2007; Schwinning, 2008; Hasselquist et al., 2010; Estrada-Medina et al., 2013; Heilman et al., 2009, 2014; Barbeta and Peñuelas, 2016; Liu et al., 2019; Carrière et al., 2020), greater water use efficiency (Hasselquist et al., 2010; Moreno-Gutiérrez et al., 2012; Nie et al., 2014; Olano et al., 2014), lower stomatal conductance and photosynthetic rates (Moreno-Gutiérrez et al., 2012; Martínez-Vilalta et al., 2014), changes in leaf morphology (Barbeta and Peñuelas, 2016), drought-deciduous (Hasselquist et al., 2010) and even slower growth (Olano et al., 2014). Thus, with the decrease of soil water availability resulted from groundwater drawdown caused by tunnel excavation in karst areas, plants could activate several physiological mechanisms and even structural mechanisms to acclimate such water deficit. The primary responses of plant species to such water stress could be the changes in water use strategies (Liu et al., 2019), including changes in the water acquisitive strategy (related to root systems) and water use efficiency (related to leaf and stem traits). Although Liu et al. (2019) found that tunnel excavation decreased the soil water contents and then changed plant water uptake patterns in karst areas, shifting from a dominant soil water source to a dominant subcutaneous water source especially in the dry season, how plants will respond remains largely unknown (is poorly understood), and the physiologic responses of plants to such water stress may be more idiosyncratic than expected, due to the complexity of karst.

Because leaf stomata can provide the most obvious mechanism allowing plants to control water transport and loss under drought conditions, stomatal closure is one of the first responses to water deficit (drought stress) (Arndt et al., 2001; Moreno-Gutiérrez et al., 2012; Martínez-Vilalta et al., 2014). A decreasing stomatal conductance results in declining photosynthesis of plants (Hasselquist et al., 2010; Sade et al., 2012), associated with the increase in water use efficiency (Hasselquist et al., 2010; Esmaeilpour et al., 2016). WUE (water use efficiency) may be estimated as the ratio between photosynthetic carbon fixation (A) and stomatal conductance (g_s), which is known as intrinsic water use efficiency ((WUEi, physiological index) (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984; Boyer, 1996; Dawson et al., 2002). Although continuous monitoring of photosynthetic carbon fixation and stomatal conductance is limited in the field (Ghrab et al., 2013; Klein et al., 2013), the time-integrated WUEi can be inferred using stable carbon isotope ratios ($\delta^{13}C$) of plant tissues, because photosynthetic carbon fixation and stomatal conductance is correlated with the ratio of intercellular to ambient CO_2 partial pressures (C_i/C_a) in C_3 plants, which reflects the balance between stomatal conductance and the photosynthetic capacity (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984). Thus, leaf $\delta^{13}C$ values can be used as a proxy for WUEi of C_3 plants based on a positive correlation between $\delta^{13}C$ values and WUEi (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984; Boyer, 1996; Dawson et al., 2002; Craven et al., 2013; Pascual et al., 2013; Esmaeilpour et al., 2016). Moreover, foliar $\delta^{13}C$ values can be used to evaluate plant response to water stress within and across species and ecosystems and provide information about the physiological performance in response to changes in the plant water status (Centritto et al., 2002; Hasselquist et al., 2010; Esmaeilpour et al., 2016). Thus, it is feasible to reveal water-use strategies of endemic species in response to tunnel excavation-induced water stress in karst areas by analyzing the changes in their leaf $\delta^{13}C$ values.

In this study, we investigated soil moisture changes at two karst trough valleys with similar hydrogeological conditions but different impacts by tunnel excavation (tunnel affected valley vs. tunnel-free valley) in Chongqing of Southwest China, and analyzed foliar carbon isotope ($\delta^{13}C$) of the four dominant woody plant species (*Fraxinus chinensis Roxb*-arbor, *Citrus reticulate*-arbor, *Viburnum chinshanense Graebn*-shrub, and *Berchemia polyphylla*-shrub) in rainy and dry seasons at two karst trough valleys in order to better understand changes in water use strategies of plants to overcome water deficit resulted from groundwater drawdown caused by tunnel excavation. We hypothesized that tunnel excavation in saturated zone lowered karst groundwater and declined soil water availability, and could result in the increasing WUEi, and then could lead to an increasing foliar $\delta^{13}C$ of woody plants in karst areas. Thus, the new aspects addressed here are to ascertain the changes in foliar $\delta^{13}C$ and water-use strategy of woody plants impacted by tunnel excavation during different seasons 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. S1). 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 watersheds are typical parallel folded karst trough valleys, located in the Guanyinxia anticline, and consisted of three ridges and two valleys, where the valleys are developed in the easily eroded Middle and Lower Triassic limestone and dolomite (T_1j and T_2l) and the ridges are formed in the erosion-resistant sandstone and shale (Lower Triassic Feixianguan Formation: T_1f and Upper Triassic Xujiahe Formation: T_3xj) (Fig. S1). The karst soil is thin, discontinuous and heterogeneous. The dominant vegetation is evergreen broad-leaf forests (*Fraxinus chinensis Roxb*, *Citrus reticulate-a fruit tree*, etc.) and shrubs (*Viburnum Chinshanense Graebn, Berchemia polyphylla*, etc.). Three about 4-km-long tunnels without any ventilation shafts, excavated below the water level of the underground rivers (saturated zone), were built within an 8-km distance of the Longfeng karst trough valley since 1999 (hereafter referred to as tunneling affected valley, abbreviated as “TAV”), resulting in lowering groundwater level gradually, drying up of surface water and epikarst springs. Contrasted, there is no any tunnel excavation in the Longche karst trough valley (hereafter referred to as tunneling unaffected valley, abbreviated as “TUAV”), and it is a natural karst hydrogeological system. Detailed information on the study areas and tunnel excavation in Longfeng karst trough valley was well described by Liu et al. (2019).

2.2. 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 station located in the Longfeng karst trough valley (Fig.

S1), were collected from January 2017 to December 2018.

Soil samples at the depths of 0–20 cm and 20–40 cm for analysis of soil water contents were collected monthly from January 2017 and December 2018. Plant foliar and atmospheric CO_2 for carbon isotopic analysis ($\delta^{13}C$) were collected in the dry season of December 2017 and March 2018, and in the rainy season of June and September 2018.

Sampling of soils and plant species was conducted from 5 cross-sections of the karst trough valleys which are the continuous limestone rock outcrops, 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. S1). There are 3 sampling points at each cross-section, of which two sampling points were located on both sides of the hillside and one 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.

At each sampling point, two soil samples were collected using the hand probes at the depths of 0–20 cm and 20–40 cm below ground. Gravimetric soil moisture content (SMC, %) was determined by drying at 105 °C in the oven for 24 h.

Fraxinus chinensis Roxb, *Viburnum chinshanense Graebn and Berchemia polyphylla,* typical aboriginal species of arbors and shrubs, and *Citrus reticulate*, a typical *fruit* tree species for karst rocky desertification restoration, were sampled at each sampling point. Two to four mature (indicated by their ability to set seed) plant individuals (one sample per individual) were randomly selected for each species, one randomly selected canopy branches were cut from each individual using high branch scissors. Ten to twenty-five 1-year-old sun-exposed leaves (depending on the leaf size) were collected from each branch. All foliage samples were oven dried at 75 °C for 48 h and then frozen in liquid N_2 and ground to a powder (pass an 80 mesh screen) for $\delta^{13}C$ analysis.

2.3. Isotopic analyses

The carbon isotopic compositions of plant foliage samples were determined using an elemental analyzer coupled to an isotope-ratio mass spectrometer (EA-IRMS). The isotopic analyses were conducted in the State Key Laboratory of Environmental Geochemistry. Stable carbon isotope results, $\delta^{13}C$, are expressed in the usual delta-notation per mil (‰) deviation from the standard Vienna Pee Dee Belemnite (V-PDB). The overall experimental accuracy for $\delta^{13}C$ measurements was ± 0.2‰.

2.4. Statistical analysis

Statistical analysis was conducted using the SPSS 20.0 program (SPSS Inc., Chicago, IL, USA). Independent-sample *t* test was used to detect significant differences in the soil moisture contents, isotopic compositions of plant foliar $\delta^{13}C$ between the tunneling affected and unaffected karst trough valleys during dry and rainy seasons and the results were considered significant at $P < 0.05$ and $P < 0.01$, respectively. The relationships between monthly soil moisture contents and plant foliar $\delta^{13}C$ values were calculated using Pearson correlation analysis, and the results were considered significant at $P < 0.05$.

3. Results

3.1. Seasonal variations of precipitation

The total precipitation was 1314 mm in 2017 and 1241 mm in 2018, respectively. More than 80% of precipitation occurred during the vegetation growing season (from April to October), accompanying with a severe summer drought in August (Fig. 1). The highest monthly precipitation usually occurred in September, while the lowest was in December. The amounts of most rainfall received between May and September were relatively large with high frequency of rainfall events. On the contrary, the amounts of most rainfall received between November and March were very small with low frequency of rainfall events (Fig. 1). Thus, plants have to experience a pronounced 5-months dry season in winter/early spring, during which < 20% of the total annual rainfall is received. Also, it was believed that plants experienced no water stress in June and September, while plants suffered from severe water deficit in November and March.

3.2. Differences in soil moisture contents between the tunneling affected and tunneling unaffected karst trough valley

As shown in Fig. 1, although the soil moisture contents of two valleys exhibited similar vertical and temporal variations, soil moisture contents in upper soil layers and lower soil layers showed significant differences ($P < 0.001$) between two karst valleys (Fig. 1). The soil moisture contents at both soil layers in the tunneling affected valley were significantly lower than in the tunnel-free valley (Fig. 1). As indicated by Liu et al. (2019), such obvious differences in soil moisture contents between two valleys suggested that groundwater drawdown resulted from tunnel excavation declined soil water availability in karst areas.

3.3. Differences in leaf $\delta^{13}C$ values of different species in the tunneling affected and tunneling unaffected karst trough valley

Leaf $\delta^{13}C$ values did not differ between arbor and shrub species at both valleys ($p > 0.05$, respectively) although leaf $\delta^{13}C$ values of arbor species showed slight higher values than shrub species (Fig. 2). Therefore, arbor and shrub species were hereafter referred to as woody plants. However, leaf $\delta^{13}C$ values of woody plants changed with the seasons at both valleys ($p < 0.01$). Leaf $\delta^{13}C$ values of woody plants were significantly higher in the dry season than in the wet season, and also showed obvious differences between two valleys at both seasons ($P < 0.001$). Similarly, leaf $\delta^{13}C$ values of woody plants were significantly higher in the tunneling affected valley than in the tunnel-free valley at both seasons (Fig. 2). As shown in Fig. 2, leaf $\delta^{13}C$ values of all species increased by about 0.95‰ and 0.75‰ from wet season to dry season in the tunneling affected and the tunnel-free valley, respectively.

Also, as shown in Fig. 2, leaf $\delta^{13}C$ values of all species increased by about 0.95‰ from the tunnel-free valley to the tunneling affected valley during monitoring periods. Therefore, the increment of leaf $\delta^{13}C$ value of woody plants caused by the tunnel excavation is almost equal to that by seasonal changes, suggesting that the decline of soil water availability resulted from tunnel excavation increased leaf $\delta^{13}C$ values of woody plants.

4. Discussion

4.1. Variations of woody plant water-use strategies revealed by seasonal differences in leaf $\delta^{13}C$ in karst areas: Rainy season vs. Dry season

All the selected four evergreen species whose foliar $\delta^{13}C$ values varying from −28.1‰ to –32.8‰ with an average of −30.2‰, are typical C_3 plants that have the $\delta^{13}C$ values varying between −20‰ and −35‰ (O'Leary, 1981). This result is consistent with the photosynthetic pathway of the dominant species in the study area, which is humid subtropical monsoon climate with the average annual precipitation of 1200 mm (Liu et al., 2019). However, mean leaf $\delta^{13}C$ values of all the four species were more positive than typical subtropical species of no-karst areas (< −30.5‰, Qu et al., 2001), indicating that they may have greater WUE. This difference resulted from the low levels of soil water availability in karst landscapes (Hasselquist et al., 2010; Fan et al., 2011; Nie et al., 2014), indicates woody plants growing on the carbonate rock outcrops have to use water more efficient than other subtropical species in no-karst areas. Meanwhile, insignificant differences in leaf $\delta^{13}C$ values between arbor and shrub

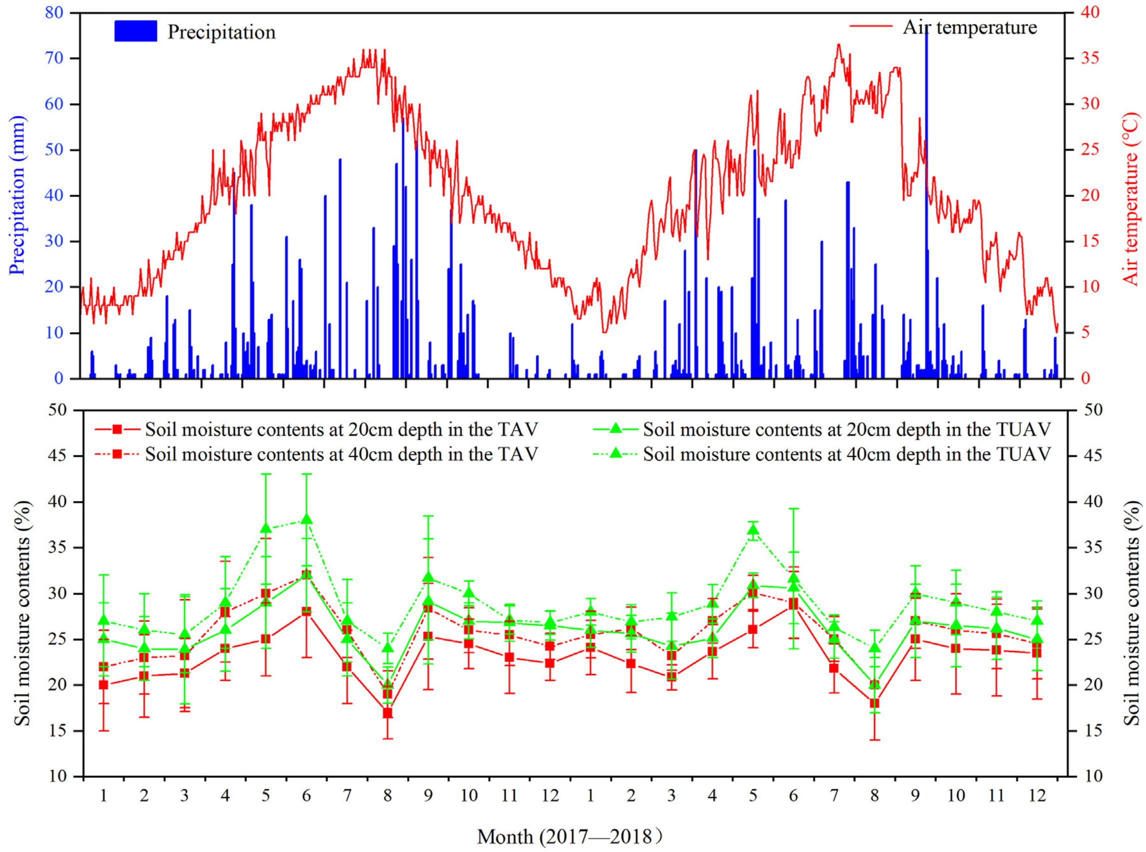

Fig. 1. Variations of daily air temperature, daily precipitation and monthly soil moisture contents during 2017 (the data from Liu et al., 2019) and 2018.

species at both valleys suggest that woody plants use same strategies to overcome water limitations in karst areas. On the other hand, as shown in Fig. 3, there are no obvious relationships between leaf $\delta^{13}C$ values of woody plants and soil water contents at different depths in both seasons, especially in dry season, suggesting that lower soil waters are not dominant water sources to support evergreen woody plants and they have access to a more permanent water source, such as groundwater. As indicated by some studies, weathered limestone bedrock often allows roots to penestrate through a network of cracks, fissures and channels to extract waters stored in epikarst and even underground river to overcome water limitations in karst areas (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; Liu et al., 2019; Carrière et al., 2020). In the study area, the proportion of water uptake from deep sources for woody plants is more than 30% in rainy season and 60% in dry season, respectively (Liu et al., 2019). The relatively positive leaf $\delta^{13}C$ values (higher WUEi) for evergreen woody plants in karst areas

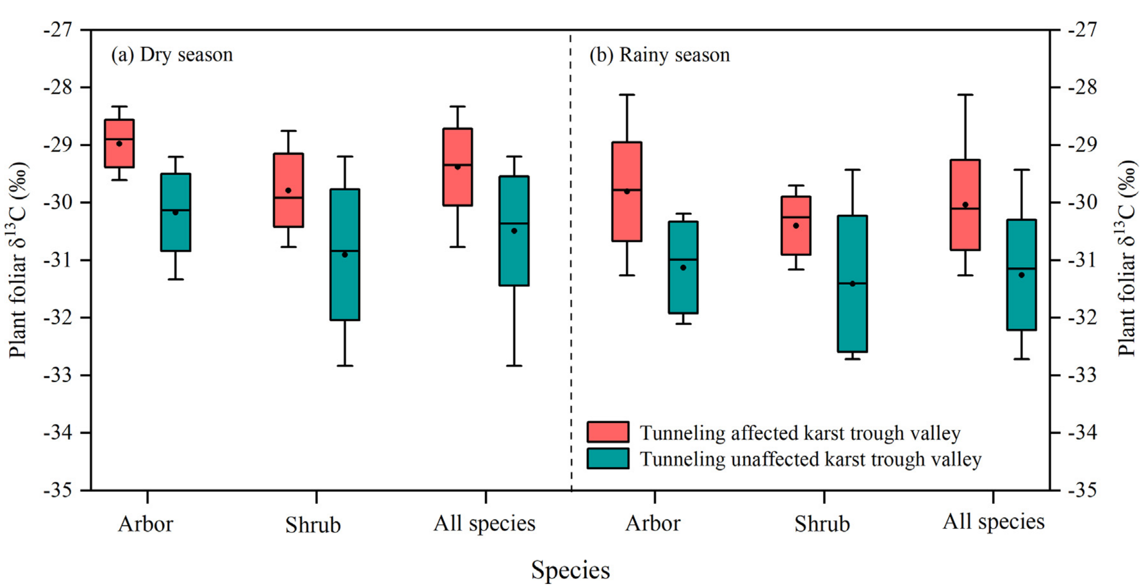

Fig. 2. Differences in leaf $\delta^{13}C$ values of arbor and shrub species in the tunneling affected and tunneling unaffected karst trough valley at different seasons (n = 9, 9, 9, 7, 18, 16, 11, 7, 7, 6, 18, and 13 from the left to the right, respectively).

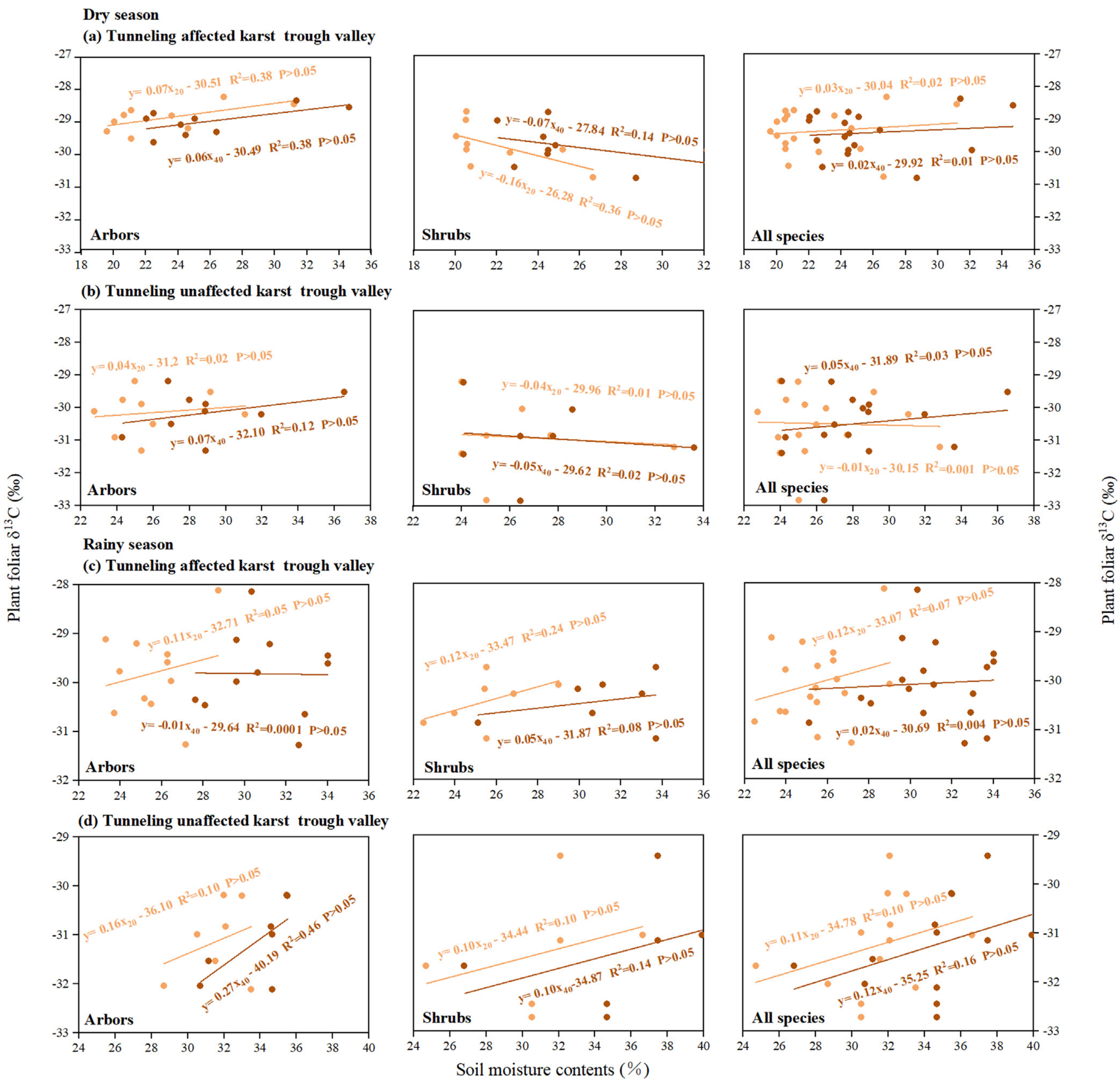

Fig. 3. Relationships between soil water contents at different depths (subscripts 20 and 40 of X represent soil depth of the upper 20 cm and the lower 20–40 cm, respectively) and leaf $\delta^{13}C$ values of arbor and shrub species in the tunneling affected and tunneling unaffected karst trough valley in different seasons.

seem to conflict with its utilization of deep-water sources. However, as indicated by some studies, the reliance on deep-water sources for plant species growing in carbonate rocky environments usually correlated with relatively positive leaf $\delta^{13}C$ values and high WUEi (Hasselquist et al., 2010; Nie et al., 2014). Thus, although plants growing on carbonate rock outcrops can rely on deep water sources, they are also dominated by conservative water use strategies in the dry season.

During the rainy season, woody plants showing profligate water-use patterns, characterized by relatively negative $\delta^{13}C$ values (lower WUEi), should be attributed to the easy accessibility of available water sources due to higher soil moisture contents resulted from lots of precipitation. As described in pervious section, a large amount of rainfall and high frequency of the rainfall events in the study area should be beneficial for karst plants to maintain more negative $\delta^{13}C$ values and lower WUEi in the rainy season. Profligate water use strategy allows plant species in karst areas to maximize photosynthesis and growth during the relatively narrow windows of opportunity when soil water availability is optimal in rainy season. However, with decrease in soil water availability in dry season, a significant increase in leaf $\delta^{13}C$ values of woody plants suggests that they suffer seriously from seasonal water stress. Plants respond to increased water stress by reducing stomatal conductance, thereby increasing leaf $\delta^{13}C$ values or decreasing carbon isotope discrimination which is interpreted as an increase in WUEi (Farquhar et al., 1989). Thus, the increased leaf $\delta^{13}C$ values (higher WUEi) of woody plants resulted from the reduced stomatal conductance during the dry season, is a more conservative water-use patterns for plants growing in carbonate rocky environments to overcome seasonal water limitations in such prolonged drought period in karst areas (Hasselquist et al., 2010; Nie et al., 2014). Therefore, such seasonal differences in leaf $\delta^{13}C$ values of woody plants should be attributed to the flexible water-use strategies, shifting from profligate water-use pattern in the wet season to conservative water-use pattern in the dry season, in different seasons depending on the soil water availability. Such flexibility of water use strategies can help plants in karst areas to overcome water limitation resulted from seasonal variations of soil water availability.

4.2. Increased leaf $\delta^{13}C$ values of woody plants resulted from tunnel excavation in karst areas: Water deficit resulted from seasonal variation of precipitation vs. water deficit resulted from tunnels excavation

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 tunnel excavation. As indicated by Liu et al. (2019), the modification of natural karst hydrogeological flow system caused by the tunnel excavation, especially drilled below underground river, could result in a rapid and great loss of soil and soil water along with the cracks and crevices developed in limestone, and consequently dry soil water contents.

With decrease of soil water contents in the tunneling affected karst trough valley at both seasons, plant foliar $\delta^{13}C$ values have been significantly increased, suggesting that plants growing in the tunneling affected karst trough valley suffer seriously from water stress. Although plants growing in the tunneling affected karst trough valley can use their deep roots to extract water from epikarst and even underground river, as indicated by Liu et al. (2019), the proportion of water uptake from deep sources for woody plants is more than 76% in dry season, it is always not easy and increases the cost of extracting water from depth for plants in karst areas (Schwinning, 2010). And even as indicated by Ryel et al. (2010), plants have evolved to use deep water pools only for maintenance functions (primarily to ensure survival) even when plenty of deep water is available. Thus, plants have to reduce stomatal conductance to control transpiration to adapt such water stress caused by tunnel excavation, thereby improving leaf-level WUEi, and then lead to an increase in leaf $\delta^{13}C$ values. Therefore, the increased leaf $\delta^{13}C$ values of woody plants in the tunneling affected karst trough valley at both seasons should be attributed to the improving WUEi resulted from the decrease of the soil water availability caused by tunnel excavation.

Furthermore, it should be noted that the increment of leaf $\delta^{13}C$ values caused by the tunnel excavation is almost equal to that resulted from seasonal change of precipitation, suggesting that such water stress caused by tunnel excavation has significantly impacted the physiological process of plants, and has the same effects on plant physiological process as the water deficit resulted from seasonal variation of precipitation in karst areas. Also, it can be concluded that improving WUEi could be a common water use strategy for native plants in karst areas during unfavorable drought conditions. Such mechanism of drought adaptation, improving leaf-level WUEi and thereby increasing leaf $\delta^{13}C$ values, can help plants in karst areas to avoid the threat of drought resulted from various factors, such as climate change, human activities, etc.

5. Conclusions

Soil water contents and leaf $\delta^{13}C$ values of woody plants were used to detect whether there were seasonal variations in soil water contents and shifts of water use strategies of plants between rainy and dry seasons in two karst trough valleys with similar hydrogeological conditions but different impacts by tunnel excavation (affected/unaffected valley) of Southwest China. Marked 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 sinificantly lower than those in the tunnel-free valley. There were significant seasonal differences in the leaf $\delta^{13}C$ values of woody plants at both valleys, showing more positive leaf $\delta^{13}C$ values (higher WUEi) in the dry season than those in the rainy season, suggesting that water use strategy for plants in rocky karst habitat shifted from profligate water-use pattern in the wet season to conservative water-use pattern in the dry season depending on the soil water availability. Furthermore, with the decline of soil water contents resulted from the tunnel excavation, leaf $\delta^{13}C$ values of woody plants have been increased at both seasons, suggesting that the water stress caused by tunnel excavation has significantly impacted the physiological process of plants in karst areas. Such flexibility of water use strategies can help plants in karst areas to overcome water limitation resulted from various factors, including climate change, human activities, etc. This study provides a useful method to explore the effects of tunnel excavation on the processes of karst eco-hydrology and ecosystem.

CRediT authorship contribution statement

Min Cao: Formal analysis, Writing - review & editing. **Chao Wu:** Resources, Investigation. **Jiuchan Liu:** Visualization, Investigation. **Yongjun Jiang:** Conceptualization, Methodology, Software, Writing - original draft, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhydrol.2020.124895.

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