



Differential stoichiometric responses of shrubs and grasses to increased precipitation in a degraded karst ecosystem in Southwestern China

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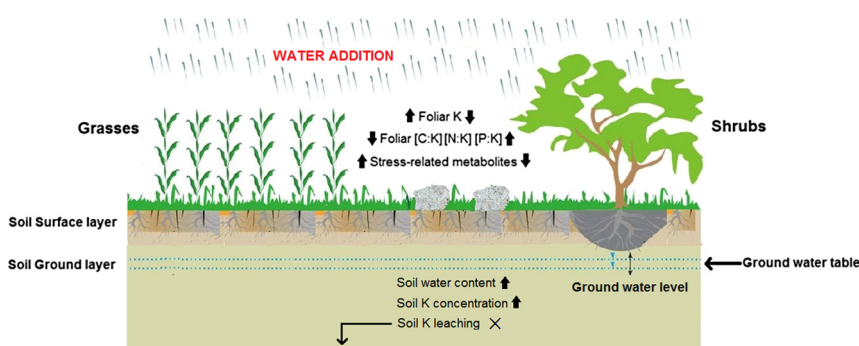
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HIGHLIGHTS

- Increased precipitation can affect the element compositions of plants and soils.
- Non-metal soil elements (C, H, N, S, and P) were relatively stable with water.
- With water addition, N and P remained unchanged in the leaves of plant species.
- K, Mg, and S decreased in shrubs and Fe, Si, and K increased in grasses with water.
- Water addition differently affected the leaf K stoichiometry in shrubs and grasses.

GRAPHICAL ABSTRACT



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ABSTRACT

The elemental concentrations of both plants and soils are sensitive to variations in precipitation due to the limiting roles of water on soil processes and plant growth in karst ecosystems of Southwestern China; however, precipitation is predicted to increase in this region. Nevertheless, it is unclear how the elemental composition of soils and plants might respond to such increases in moisture. Particularly, how potassium (K) may behave as a key mediator in the regulation of the water potential of plants. For this study, the responses of the elemental composition of both soils and plants to the variable addition of water were investigated. Two grasses (*Cymbopogon distans* and *Arundinella setosa*) and two shrubs (*Carissa spinarum* and *Bauhinia brachycarpa*) were investigated under four levels of watering treatments 0%, +20%, +40%, and +60%, relative to the annual rainfall, respectively. Compared to the control (CK), the soil water content (SWC) increased to 3.75, 3.86, and 4.34 mg g⁻¹ in T1, T2, and T3 groups, respectively ($p < 0.05$). Non-metal elements (C, H, N, S, and P, except for Si) in the soil were relatively stable with water addition; however, metal elements (Al, Na, Mg, Fe, and K, along with Si) increased significantly, whereas Zn and Ca decreased ($p < 0.05$). With water addition, leaf N and P remained unchanged in all four species, while K, Mg, and S decreased in both shrubs (higher C:K, N:K, and P:K). Increases in Fe, Si, and K were observed in both grasses (lower C:K, N:K, and P:K), which suggested that K played distinct roles for water regulation in shrubs and grasses. These findings implied that the

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elemental compositions of both soils and plants might be altered with increasing precipitation in the future, where different plant types may adopt distinct K-regulation strategies to cope with variable soil moisture.

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1. Introduction

Global climate change, whether caused by anthropogenic activities, or natural processes, or a combination of both, may have considerable effects on global hydrological cycles, which are likely to result in increased precipitation, higher evaporation rates, and the uneven distribution of rainfall on a global scale (Lian et al., 2015; Nijssen et al., 2001). Global climate change is considered to be one of the major drivers of changes in natural ecosystem. Particularly, changes in precipitation influence the composition of plant and soil communities, and thus impact the functionality and services of natural ecosystems (Anderson-Teixeira et al., 2013; Cregger et al., 2012). Global warming poses a severe threat to karst hydrogeology and affects the magnitude of soil organic carbon in the karst areas of Southwest China (Piao et al., 2000). Because climate change may have a considerable effect on karst hydrological cycles, it is essential to accurately elucidate the effects of altered precipitation on the elemental composition and their key processes in these ecosystems.

Karst topographies are formed by irrational and intensive land use that results in the geocological destruction of fragile karst environments (Wang et al., 2004; Yan and Cai, 2015). Extensive land degradation and deterioration of vegetation in karst regions cause severe drought and soil erosion, which creates a rocky-like landscape (Wang et al., 2004). Massive degraded karst plant communities exist in the rocky areas of Southwestern China due to geological conditions and strong human disturbances such as urban intensification, agricultural extension, livestock grazing, and firewood production (Liu et al., 2009). Across these vegetation-degraded areas, there is the very slow formation of soil derived from underlying CaCO₃, shallow and porous soil, and extremely heterogeneous microhabitats (Liu, 2009; Liu et al., 2009; Zhu, 1997). In fact, ground soils in karst ecosystems continuously experience long-term dry periods in the drought season followed by a rapid wetting during the rainy season. Consequently, species in degraded karst plant communities adapt to drought with high levels of calcium through long-term evolution (Daoxian, 2001; Wu et al., 2011). Based on multiple studies, the annual precipitation in Southwestern China is predicted to increase (Piao et al., 2010; Qin et al., 2015). However, the responses of karst soils and plants to increasing precipitation remain unclear.

Plants obtain nutrients entirely from recycling processes in both natural and semi-natural ecosystems, such as the decomposition of organic matter, along with minor inputs from the atmosphere, and the weathering of soil minerals (Whitehead, 2000). Climate change impacts nutrient availability in soils and modifies the physiology of plants, while altering their stoichiometry, which affects plant growth and development (Peñuelas et al., 2008; Sardans et al., 2008). Therefore, leaf stoichiometry is a key parameter in the study of plant nutrient limitations, nutrient cycling, and plant responses to climate change (Austin and Vitousek, 2012; Baxter and Dilkes, 2012; Güsewell, 2004). Furthermore, various elements are critical for proper plant growth and nourishment under stressed conditions (Uchida, 2000). Some macroelements are typically required in large amounts (e.g., N, P, K, S, Mg, and Ca), while other microelements are required in very low quantities (e.g., Zn, Fe, Mn, Mo, and B) (Zhou et al., 2015).

Stoichiometric homeostasis, which is a key measure of plant stoichiometry, describes a plant's ability to maintain its stoichiometric ratios around a given species- or stage-specific value, despite variations in the relative availability of elements in its resource supplies (Koojiman, 1995). The C:N:P stoichiometric ratios of an organism could be considered its elemental phenotype, which have been shown to be essential for many ecosystem processes. These include litter decomposition (d'Annunzio et al., 2008), N₂-fixation capacity (Sañudo-Wilhelmy et al., 2001), plant-herbivore-predator relationships (Kagata and Ohgushi, 2006; Ngai and Jefferies, 2004), specific-ecosystem diversity and composition (Olde Venterink et al., 2003; Roem and Berendse, 2000) and the capacity of plants to adapt to climatic stress (Sardans and Peñuelas, 2007; Woods et al., 2003). Furthermore, Rivas-Ubach et al. (2012) described the strong relationship between the foliar C:N:P:K stoichiometry and leaf metabolome, which plays a significant role in plant growth and development under environmental stress.

Water availability is the key limiting factor in the functionality of desert and grassland ecosystems (Gherardi and Sala, 2013). Consequently, several researchers have studied the effects of moisture variations in soils to understand its impacts (Fay et al., 2008; Knapp et al., 2008). In this context, manipulated-precipitation experiments are considered as the principal tool to better understand the consequences of climate change on the functioning of ecosystems (Leuzinger et al., 2011). However, such experimental analogs are neither realistic nor equivalent to the effects of long-term climate change (Beier et al., 2012). In the meantime, the rainfall patterns of Southern China have become more concentrated over the last decade (Piao et al., 2010; Qin et al., 2015), and climate change is predicted to intensify rainfall patterns (Sternberg et al., 2011). Therefore, manipulated-precipitation experiments may be employed to study such predicted behaviors in degraded karst areas.

As mentioned above, water conditions comprise one of the essential factors that control the dynamics and stoichiometry of elements in karst ecosystems. In this study, we conducted a manipulated-precipitation experiment in a shrub- and grass-dominated community in a typical karst area of Southwestern China to mimic expected precipitation scenarios. The queries we endeavored to address were:

- i) How elemental concentrations of soils altered with water addition in such degraded karst areas?
- ii) How drought-adapted plants respond to increasing precipitation in terms of elemental concentrations and K stoichiometry.

2. Materials and methods

2.1. Study area

This study was based on a field experiment located at the Karst Ecosystem Research Station (KERS) in Jianshui County (23° 59' N, 102° 93' E), Yunnan Province, in Southwest China, which was conducted with a mixed community of natural shrubs and grasses (Fig. 1). The climate is typically in a subtropical monsoon category with two different seasons; a dry season (December to May) with

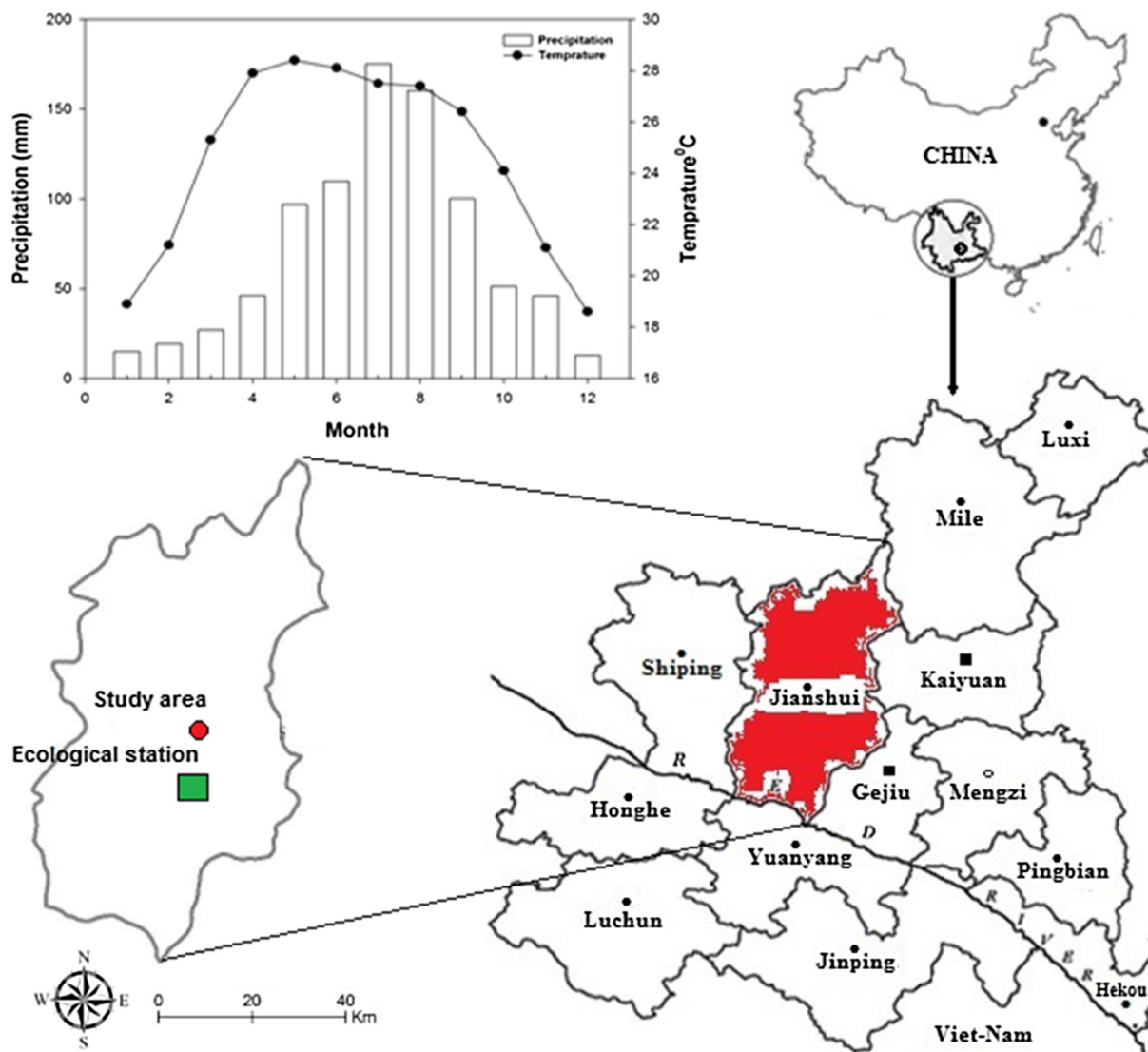


Fig. 1. Location of the study area in Jianshui County, southeastern Yunnan, southwestern China. The distribution of annual precipitation and temperature from (2010 to 2017) was obtained by reference to nearby meteorological observation stations.

15% of annual rainfall, and a wet season (June to November) with 85% of annual rainfall. The long-term average rainfall is 700 mm (1981–2010), with an average precipitation of about 17.6% between April and May (<http://data.cma.cn/en/?r=data/weatherBk>). The annual average temperature of this region is 20 °C, with the average minimum at 14.6 °C and the average maximum at 24.6 °C, while the median relative humidity is 71.8% (2010–2017). The average pH of the surface soil is 6.3 (1 M KCL) (Umair et al., 2019).

Due to anthropogenic disturbances, soil and vegetation degradation are key issues in the generation of large areas of bare lands in Southwestern China. The natural vegetation of the surrounding plains comprises subtropical evergreen broad-leaf forests with specific flora, such as *Arundinella setosa*, *Barleria cristata*, *Bauhinia brachycarpa*, *Carissa spinarum*, *Cymbopogon distans*, *Dodonaea viscosa*, *Elsholtzia ciliate*, *Myrsine africana*, *Osteomeles anthyllidifolia*, and *Pinus yunnanensis*. In this degraded area, drought-tolerant shrubs and grasses are prominent plants (e.g., two deciduous bushes, *Bauhinia brachycarpa* and *Carissa spinarum*, and two perennial grasses, *Arundinella setosa* and *Cymbopogon distans*).

2.2. Experimental design

Manipulated-precipitation experiments are typically designed to analyze the rate of change that leads to a step change in climatic drivers (Beier et al., 2012). We employed a randomized complete block design (RCBD) that consisted of five sub-blocks, which was conducted in 20 sample plots (3 × 3 m in size) (Fig. S1). Each plot was separated from its neighboring plot by a distance of 3 m. Zhang et al. (2019) described the correct methods for measuring precipitation, and designed the experiment for examining the impacts of increased precipitation on soil and plant ecosystems.

In this manner, we designed four watering treatments (CK (control), T1, T2, and T3), which translated to 0%, 20%, 40%, and 60% increases, respectively, relative to the average monthly precipitation. The average monthly rainfall was measured on the basis of 2010–2017 climatic data from a nearby meteorological station (Fig. S2). During the study period, the climatic data revealed that there was an average of 25 annual rainfall days (Fig. S3). The median annual duration of sunlight was 426 h during the study period. Our study was conducted during the dry season; thus it is likely that the sunlight hours were longer during this particular time.

2.3. Measurements of precipitation, plant, and soil sampling

The natural precipitation was 143 mm, from the beginning of April to the end of May in 2017 during the growing season (Fig. S3). In the karst region of Southwestern China, the growing season spanned from beginning of April to October (Hou et al., 2015). The water addition treatments were set up through the addition of natural precipitation, and the exact values of artificial rainfalls were 172 mm for T1, 200 mm for T2, and 228 for T3. The control treatment blocks received ambient quantities of rainfall. During the growing season, the additional precipitation was manually sprayed onto the related plots three times each month, in the mornings of days 10, 20, and 30. Water was slowly and evenly sprayed over each plot such that run-off from the plot was avoided.

For this study, two dominant grasses (*Cymbopogon distans* and *Arundinella setosa*) and shrubs (*Carrisa spinarum* and *Bauhinia brachycarpa*) were selected as study plants, and each occurred in all of the sampling plots (Table 1). *Cymbopogon distans* was the most dominant species at the study site, which is a tall (up to 90 cm) and productive grass with medicinal importance in the karst limestone mountainous areas, or hot dry river valleys of Southern China. *Arundinella setosa* is a drought-resistant grass found in open forests, forest edges, and grassy hillsides (200–2300 m) of the South China Karst.

The thorny shrub "*Carrisa spinarum*" is found wild in Southern China, particularly in the dry mountain slopes of the karst regions at up to elevations of 1,500 m. It is a tall (up to 5 m) and highly drought-resistant species. *Bauhinia brachycarpa* is a drought-tolerant species with small leaves and deep roots that dominates in the dry karst ecosystem of Jianshui County, in Yunnan, China.

At the end of May 2017, plant leaves were sampled in all plots. From each plot, the compound samples of five plants of each selected species were obtained. Fresh green leaves were uniformly selected in the middle of the canopy from the four species under study. The plant samples were placed in a tank filled with liquid nitrogen, and then transferred to the analysis center using dry-ice box method. In the analysis center, the plant leaf samples were washed with distilled water and dried at 65 °C for 72 h in the oven box. The dried samples were ground, sifted through a 1-mm mesh sieve and stored for analysis.

When the leaf samples were obtained, soil samples were also extracted at four sites of each plot using a soil corer (Ø2.5 cm × 0–10 cm depth, excluding the litter layer) and mixed to form a composite sample. The composite soil sample was placed into a plastic bag for chemical analysis. Meanwhile, additional soil samples were collected and sealed in aluminum tins for moisture measurements. All samples were appropriately packed and transported to the laboratory.

2.4. Soil water content and pH measurement

A digital pH meter was employed to measure the soil pH in 1:5 soil/1 M KCl solutions (Mettler Toledo FE20/EI20, Shanghai, China). The soil water content (SWC) was calculated using an oven-drying method. The soil samples were oven-dried for 48 h at 105 °C and the SWC was calculated by using the following formulae:

$$SWC = \frac{W1 - W2}{W2 - W3}$$

where W1 is the weight of wet soil plus box, W2 represents the weight of dry soil plus box, and W3 represents the box weight.

HOBO® Micro Station Data Loggers (H21-002) were installed to check the soil temperature (°C) and volumetric soil moisture content (m³/m³) at the end of dry season. The soil moisture conditions were monitored using an automatic soil moisture smart sensor (HOBO Micro Station #H21-002 Data Logger; Onset Computer Corp., Bourne, MA, USA; #S-SMx-M005) at the end of dry season (Fig. S4). To measure the soil temperature, HOBO 12-bit temperature smart sensors (S-TMB-M0xx) were connected to HOBO Micro Station data loggers. Automatic measurements were taken every 10 s (Fig. S5). All measurements were recorded by a HOBO® Micro Station Data Logger.

2.5. Elemental analysis

The concentrations of C, N, H, and S were determined using 25 mg of each sample using an isotope ratio mass spectrometer (Vario EL III Element Analyzer; Elementar, Germany). To quantify the Fe, P, K, Mn, Mg, Ca, Na, Al, Cu, and Si the samples were digested in a microwave reaction chamber under high temperature and pressure. A 250 mg quantity of sample powder was transferred to a Teflon tube with 2 mL of HNO₃ and 1 mL of H₂O₂. A Digiprep-MS digestion block (SCP Science, Champlain, NY, USA) was employed for the digestion of the samples. The digested samples in 50 mL flasks were refilled with distilled water to a 40 mL volume. The elemental concentrations were measured using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo Jarrell Ash IRIS Advantage 1000, Franklin, Massachusetts) at the Testing Centre of Shanghai Jiao Tong University, China.

2.6. Calculating correlation coefficients

Bivariate correlation measures the strength and statistically quantifies the linear association between two component variables. The regulation ability was estimated by calculating the regulation coefficients for each element from soil source and leaf element concentrations. We excluded the studies of element ratios

Table 1
Description of target species in the study area.

Species and family name	English name	Flowering period	Growth form	Description
<i>Carissa spinarum</i> L. (Apocynaceae)	Conkerberry	March to May	Evergreen shrub	Evergreen spiny shrub up to 5 m in height, occurring on hilly slope. The branches bear thorns of 1–3 cm length and highly drought resistant species.
<i>Bauhinia brachycarpa</i> Wall. ex Benth. (Fabaceae)	Faber bauhinia	May to July	Evergreen shrub	Evergreen shrub up to 5 m, occurring on dry mountain slopes. It has dual lobed leaves on twigs
<i>Cymbopogon distans</i> (Nees ex Steud.) Wats (Poaceae)	Remote lemongrass	June to October	Perennial grass	Perennial aromatic grass upto 50–100 cm tall, occurring on dry mountain slopes. The essential oils are extracted for medicinal and industrial purposes.
<i>Arundinella setosa</i> Trin. (Poaceae)	Reed grass	August to December	Perennial grass	Perennial grass tufted from a short rhizome, occurring on grassy hillsides. The culms are slender or stout upto 50–100 cm long and the leaves are occasionally hairy.

to the compare homeostatic regulation between individual elements. The r^2 was also measured to calculate the variability and cross relations between the soil and leaf element concentrations. The degree of stoichiometric relationships was described via the regulation coefficient (H^{-1}), explained by Sterner and Elser (2002).

$$H^{-1} = \frac{\ln(y) + \ln(C)}{\ln(x)}$$

where y is the concentration of elements in plants, x is the concentration of elements in the soil, c is a constant, and H^{-1} is the slope of the log-linearized relationship. The H^{-1} values were calculated from the linear relationships between x and y through regression analysis with PAST 3.20 (Hammer et al., 2001). A lower H^{-1} represents a strictly stable regulation, while a higher H^{-1} shows plasticity. The degree of regulation coefficient of an element is strictly stable, when regression $p \geq 0.1$, 'stable' when $0 < 1/H < 0.25$, 'weakly stable' when $0.25 < 1/H < 0.5$, 'weakly plastic' when $0.5 < 1/H < 0.75$, and 'plastic' when $1/H > 0.75$ and regression $p < 0.1^2$.

2.7. Statistical analysis

A one-way ANOVA was performed using SAS version 9.0, which determined the effects of watering treatments on the soil and leaf element concentrations. We employed Duncan's Multiple Range Test with LSD ($p < 0.05$) to identify the significant difference for mean comparison. We also used Dunn's post-hoc tests to calculate the statistical significance ($p < 0.05$) between four watering treatments. An analysis of covariance (ANCOVA) was performed to find the variations between the control and water-treated samples. The data was \log_e transformed for the analysis of covariance (ANCOVA) and linear regression. The linear regression model was used to explain a best fit line for a linear relationship between the soil and leaf element concentrations. In addition, principal component analysis (PCA) was conducted to explain whether the soil elemental variables between the four watering treatments could be discriminated, and to evaluate how SWC effected the elemental composition of the soil.

We also used multi-variate PCA to discriminate the patterns of water-treated samples in shrub and grass community variables during the growing season. The significance of PCA scores was confirmed by two-way PERMANOVA using the Euclidean distance, to learn the significant differences between plant species. Pearson correlation analysis was performed to find the correlation between

the elemental compositions of the leaves and soil. Statistical analyses were performed using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA), while graphical data were analyzed using PAST 3.20 (Hammer et al., 2001), Sigma-Plot 10.0 (Systat software, Inc., 2006).

3. Results

3.1. Variations in the elemental concentrations of karst soil

The results revealed significant variations in SWC between the control and various water treatments (ANOVAs, $p < 0.001$). Compared to CK, the SWC increased by 375, 386, and 434 mg g^{-1} , respectively, in T1, T2, and T3 (Table S1). Between the 15 elements, the concentrations of K, Ca, Mg, Na, Fe, Al, Mn, Zn, Cu, and Si differed significantly between treatments ($p < 0.05$), while no significant change was observed for the other elements (Table S1).

Variations in elemental concentrations were observed to decrease with higher SWC in a different manner for all treatments i.e., CK (regression slope = -0.82 , $p = 0.44$), T1 (regression slope = 2.76 , $p = 0.25$), T2 (regression slope = -0.009 , $p = 1.00$) and T3 (regression slope = -3.39 , $p = 0.039$) (Fig. 2). This homogeneity (or equality) of slope significantly differed between CK and T1 (ANCOVA, $pseudo-F = 4.267$, $p = 0.04$); however, it was approximately the same for CK and T2 (ANCOVA, $pseudo-F = 0.06$, $p = 0.8$), and CK and T3 (ANCOVA, $pseudo-F = 0.90$, $p = 0.35$). The soil K, Mg, Na, Fe, Al, Si, Cu, and Mn concentrations (except for Ca and Zn) increased significantly with higher SWC, which resulted in a decrease of the variation of soil element concentrations.

As shown in Fig. 3A, there were significant differences in the element concentrations of soils between the control and water-treated samples, separated along the PC1-axis (explaining 56.7% variation, $p < 0.001$). The soil elements in T2 (green triangle on the right) were significantly separated from CK along the PC1-axis; however, this was not the case for T1 ($p < 0.05$). The T3 samples were primarily positioned at a positive direction along the PC1-axis, while the control samples were mainly scattered in the negative direction (Fig. 3A).

The PC1-axis (explaining 56.7% of the variation), mainly containing soil Fe, K, Mg, Na, and Al, was positively correlated with SWC ($p < 0.05$), while the PC2-axis (explaining 24.7% of the variation), mainly containing soil N, C, H, S, P, Ca, Mn, Zn, Cu, and Si had no consistent relation with SWC ($p > 0.05$) (Table S2). All these

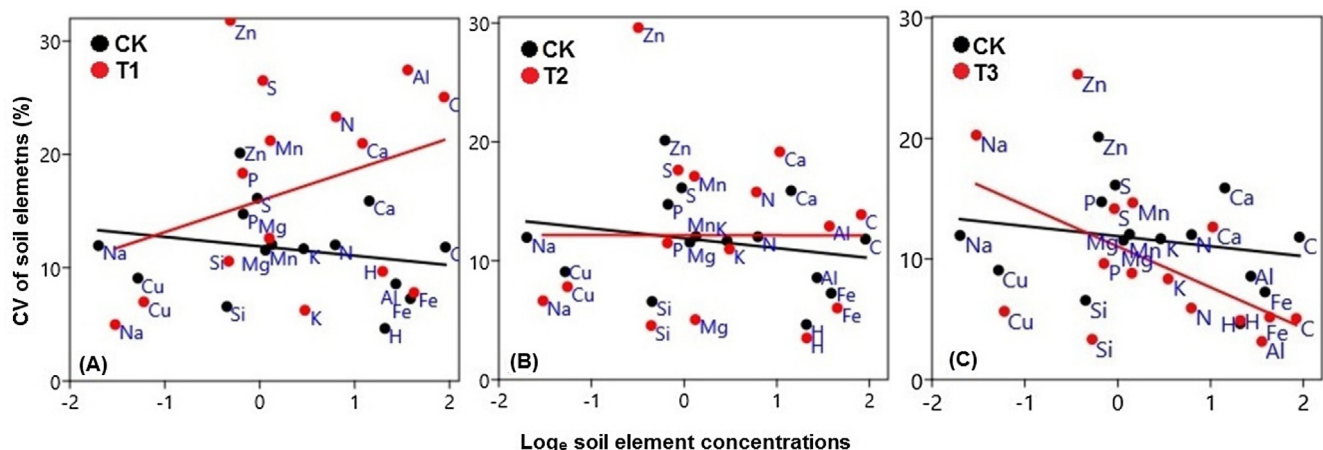


Fig. 2. Relationships of soil element concentrations and their coefficients of variation (CV%) between (A) CK (0%) and T1 (+20%), (B) CK and T2 (+40%) and (C) CK and T3 (+60%). Watering treatments are indicated by different colors (black circles, CK; red circles, T1, T2 and T3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

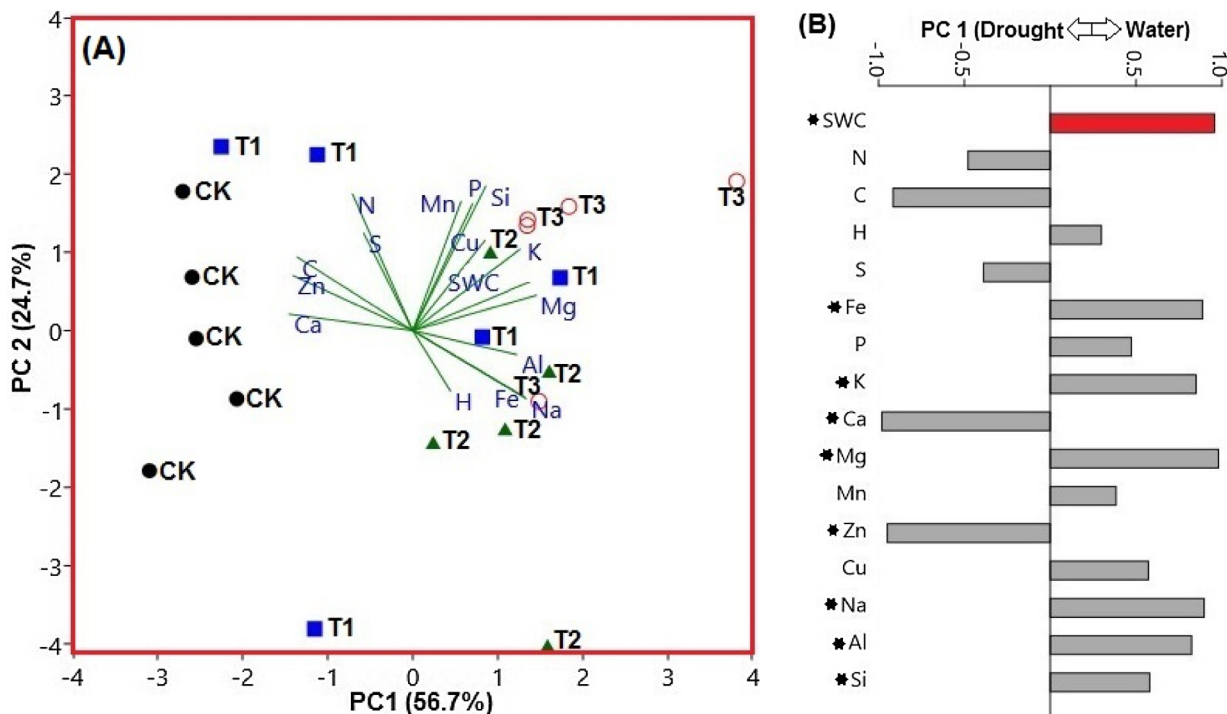


Fig. 3. (A) Plot of variables in the PCA conducted with SWC and soil elements under the 0% (CK), +20% (T1), +40% (T2) and +60% (T3) watering treatments. Watering treatments are indicated by different colors and geometrical figures (black circles, CK; blue squares, T1; green triangles, T2; red circles, T3). The length of green line indicates the overall contribution of the element to the analysis. The direction of green line indicates element correlation with each axis (vector lines parallel to an axis are highly correlated with that axis). Angles between vector lines show correlations among elements. (B) Loadings of SWC and elemental variables in PC1 separating drought and watering treatment. The soil variables are represented by different colors: red, soil water content (SWC); dark gray, soil elements. Asterisks showed statistical significance ($p < 0.05$) in one-way ANOVAs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

elements were positively correlated with SWC except for N, C, S, Ca, and Zn (positive loading coefficients on PC 1) (Fig. 3B).

3.2. Effects of water addition on elemental concentrations in shrubs and grasses

The concentrations of K, S, and Mg decreased with water addition in the leaves of the two shrubs (Fig. S6). Compared to CK, the concentrations of S, K, and Mg for T3 decreased by 40.4%, 27.9%, and 21.9% in *C. spinarum* and 18.1%, 25.0%, and 3.03% in *B. brachycarpa*, respectively (Table S3). In contrast, the concentrations of Fe, Si, and K significantly increased in both grasses with water treatments (Fig. S7). Compared to the CK, the Si and K concentrations for T3 increased by 140% and 51.6% in *A. setosa*, and 27.4% and 22.4% in *C. distans*, respectively (Table S4). However, shrub leaves had the highest N:K and C:K ratios and the lowest K:P ratio (Fig. S6), while grasses had the lowest N:K and C:K ratios and highest K:P ratio with increasing moisture from CK to T3 (Fig. S7).

Leaf K, Mg, and S concentrations decreased in both shrubs, while leaf Fe, Si, and K increased in both grasses with water addition (Tables S2 and S3). The overall elemental composition usually varied in *C. spinarum* and *B. brachycarpa* with increased water supplies (Fig. 4A). PCA scores showed that the leaf stoichiometry differed significantly, depending on the species at PC1 (explaining 63.6% variation; $pseudo-F = 223.3$; $p < 0.01$) and watering treatments at PC2 (explaining 18.8% variation; $pseudo-F = 5.269$; $p < 0.01$) (Table S5). This indicated that plant species were the primary factor, while water treatment was the secondary factor for plant leaf stoichiometry.

However, the elemental composition of both shrubs shifted toward lower concentrations of K, Mg, S, and K:P ratio, and higher

C:K and N:K ratios with increasing water additions (Fig. 4C). The PCA of elemental and stoichiometric variables showed that *C. distans* and *A. setosa* were separated along the PC1-axis (explaining 67.4% of the variation, $pseudo-F = 113.6$; $p < 0.01$) (Fig. 4B and Table S6). The elemental composition of two grasses shifted toward higher concentrations of Fe, Si, K, and K:P ratio, as well as lower C:K ratio with increasing water supplies (Fig. 4D).

3.3. Regulation coefficient for elemental concentrations in leaves with the availability of elements in soils

Leaf K and Mg concentrations were negatively correlated with their soil counterparts, whereas leaf Ca and Zn concentrations showed a positive association with soil elements in *C. spinarum* (Table S7). In the case of *B. brachycarpa*, leaf Si, Fe, and Mn (except Na) concentrations were positively correlated with their soil counterparts (Fig. S8). There were significant correlations in leaf Si, K, and Zn concentrations in the two grass species with those in soils; however, leaf Mg exhibited different relations with their soil counterparts (Fig. S9 and Table S8).

The regulation coefficient (H^{-1}) was calculated on the basis of a regression relationship in the elemental concentrations between leaves and soils (Fig. 5). Most elements were strictly stable in *C. spinarum* leaves except for K (plastic), Ca (weakly plastic), Mg (plastic), and Zn (weakly plastic). In *B. brachycarpa*, leaf Na, Fe, Mn, and Zn showed plasticity, while the other elements were strictly stable. The results revealed that leaf K, Mg, Zn, and Si were not strictly stable in both grass species, whereas leaf S showed plasticity only in *A. setosa* (Table 2). In this study, the regulation of macroelements (especially N and P) was strictly stable in contrast to the other elements in all four species.

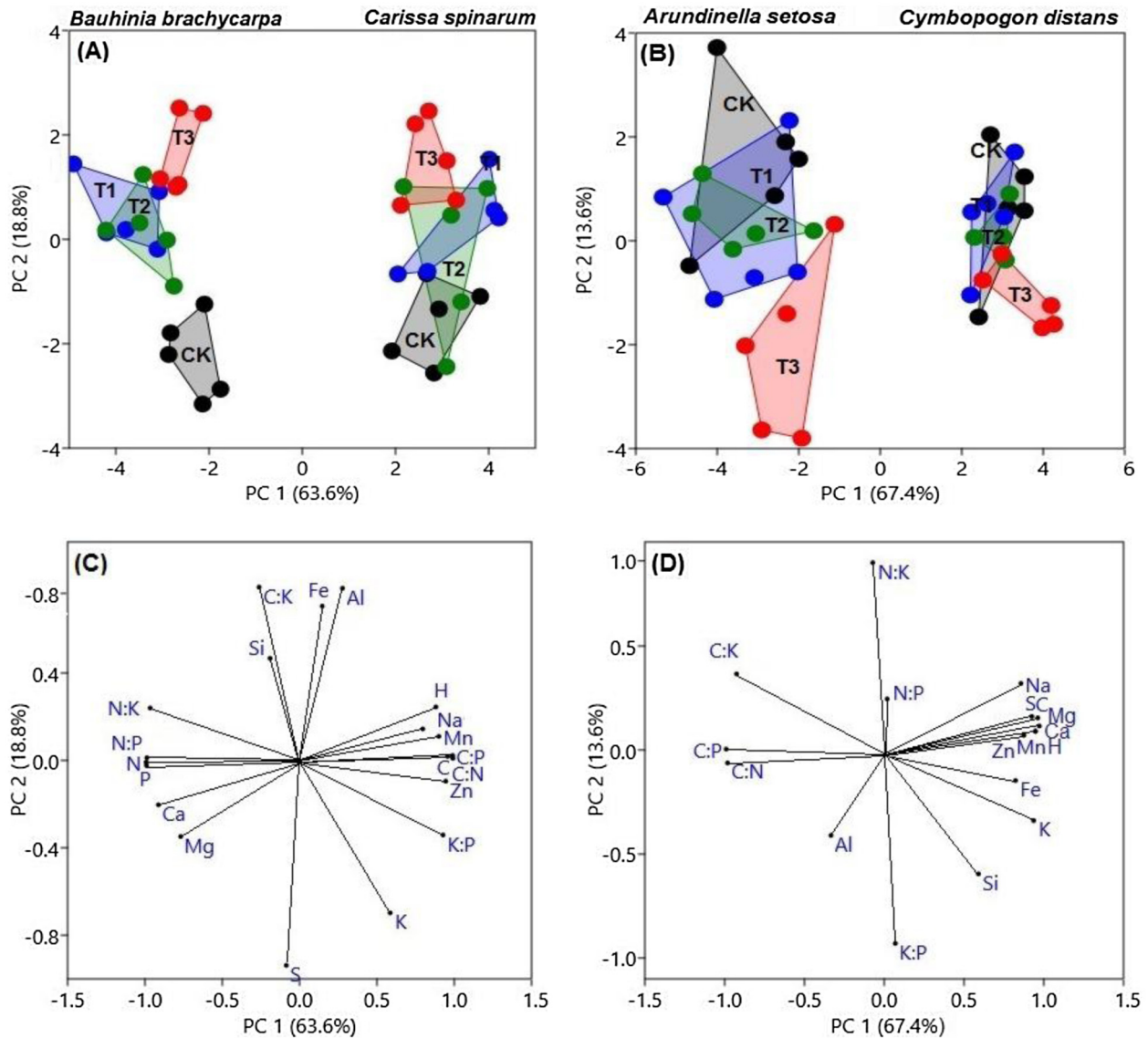


Fig. 4. PCA plots of the scores of first two PCs for the A) shrubs and B) grass species under the 0% (CK), +20% (T1), +40% (T2) and +60% (T3) watering treatments. Watering treatments are indicated by different colors (black circles, CK; blue circles, T1; green circles, T2; red circles, T3). Percentages within brackets in the X and Y axes legends indicate the percentage of variance explained by the corresponding PC. The corresponding variable distributions in C) shrubs and D) grasses are shown. The length of black line indicates the overall contribution of the element to the analysis. The direction of black line indicates element correlation with each axis (vector lines parallel to an axis are highly correlated with that axis). Angles between vector lines show correlations among elements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Sensitivity of soil metal elements to water addition in degraded karst areas

Our results clearly revealed that non-metal soil elements (C, H, N, S, and P, except for Si) remained almost unchanged with water addition; however, metal elements (Al, Na, Mg, Fe, Cu and K, along with Si) were significantly increased, while the metal elements Zn and Ca decreased. This is consistent to Wang et al. (2016) study, increased precipitation led to decreased concentrations of most elements (Ca^{+2} , Mg^{+2} , SO_4^{+2} , HCO_3^-) with the exception of K^+ and Na^+ in the karst areas of Southwest China. Such patterns of elemental variations might characterize the chemical weathering of silicate rocks (granites and metamorphic rocks), due to increased precipitation, is one of the key natural processes in the production of K^+ and Na^+ in the subtropical karst basin, of Southern China (Lyu

et al., 2018). According to a report by Wang et al. (2018) soil C, N, and P in estuarine wetlands showed no obvious change with flooding intensity, but differed significantly with soil depth. With two-year watering treatment had no significant effect on soil extractable N and P in tropical forests (Wang et al., 2019). However, in a report by Misra and Tyler (1999), with soil moisture increases from 35 to 85%, relative to a control in a greenhouse, soil P, Mn, and K concentrations increased along with pH and HCO_3^- , while Ca, Mg, and Zn concentrations decreased. This reflected the characteristics of different elements to describe variations in the soil moisture of karst areas, which were distinct from those in greenhouse experiments and under in situ conditions.

Variations in elemental concentrations are controlled by multiple factors; however, in the context of moisture variations, the main processes involved in the release and fixation of soil nutrient elements include precipitation-dissolution and desorption-adsorption (Singh and Schulze, 2015). Elements in soil solutions

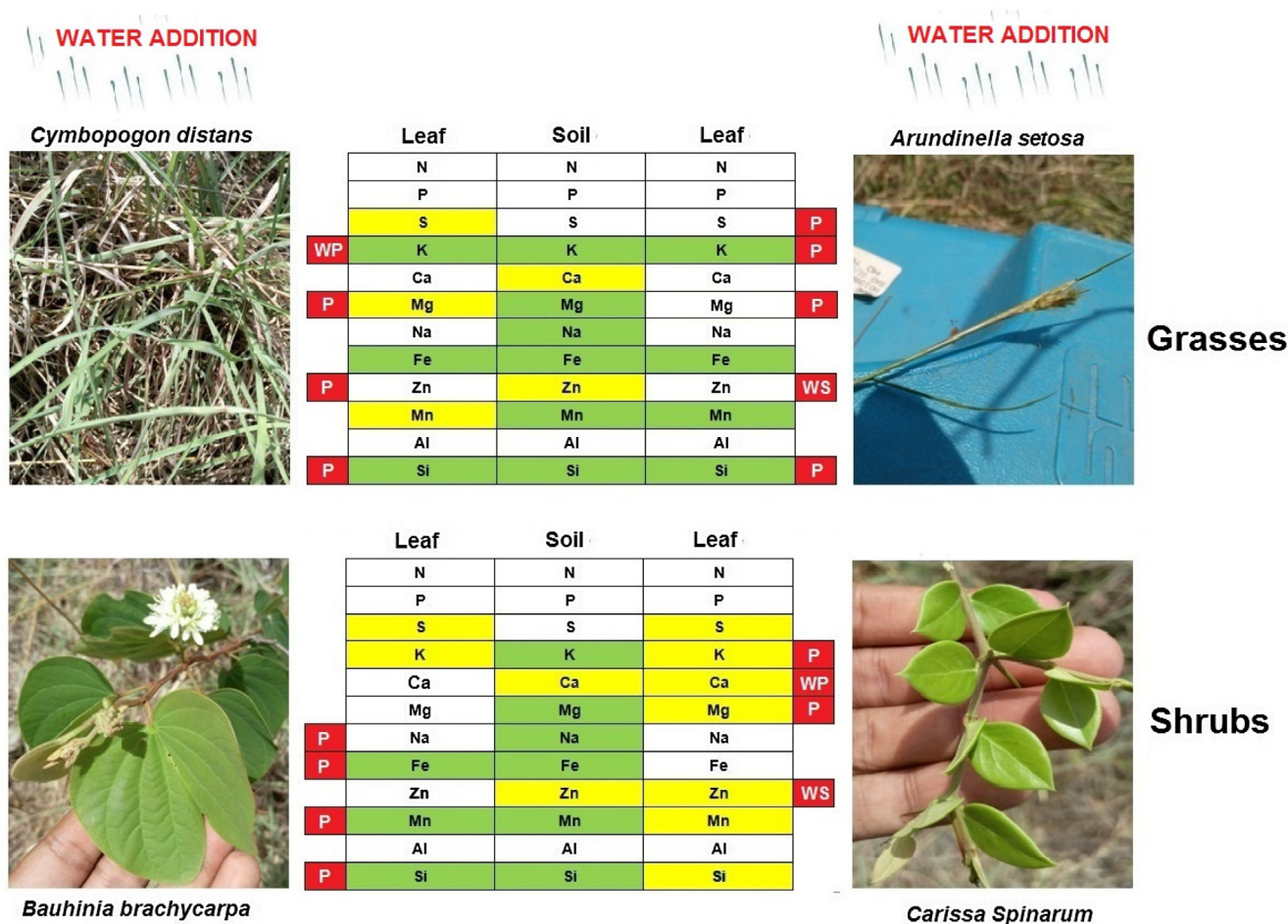


Fig. 5. Graphical representations of the elemental relationship between leaves of four plant species and soil supply. Green and yellow colors show increased and decreased element concentrations, respectively. The red color cell indicates significant regulation coefficients (H^{-1}) of the elements. The plasticity of elements was categorized into different groups: plastic (P), weakly plastic (WP) and weakly stable (WS). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Regulation coefficient (H^{-1}) and standard error (\pm SE) of leaf elements in studied species in all sampling sites ($n = 20$). The values in bold indicate statistical significance at $p < 0.05$.

Elements	<i>C. spinarum</i>			<i>B. brachycarpa</i>			<i>C. distans</i>			<i>A. setosa</i>		
	H^{-1}	SE	p	H^{-1}	SE	p	H^{-1}	SE	p	H^{-1}	SE	p
N	0.047	0.094	0.625	0.035	0.142	0.808	0.029	0.164	0.862	0.024	0.279	0.933
P	0.131	0.182	0.483	0.024	0.169	0.921	0.075	0.156	0.635	0.191	0.332	0.573
S	0.225	0.298	0.459	0.148	0.278	0.601	0.025	0.263	0.961	0.884	0.317	0.012
K	0.754	0.351	0.045	–	–	–	0.57	0.281	0.033	1.035	0.48	0.045
Ca	0.543	0.255	0.047	0.103	0.169	0.55	0.026	0.19	0.894	0.048	0.251	0.851
Mg	0.79	0.34	0.032	0.155	0.287	0.596	0.798	0.296	0.015	1.046	0.433	0.027
Na	0.376	0.521	0.479	1.609	0.499	0.005	0.055	0.416	0.896	0.332	0.402	0.42
Fe	0.622	0.605	0.318	1.889	0.85	0.039	0.173	0.854	0.842	0.046	0.646	0.944
Zn	0.23	0.106	0.043	0.05	0.095	0.604	0.207	0.094	0.041	0.424	0.184	0.033
Mn	0.044	0.362	0.906	0.804	0.363	0.04	0.397	0.355	0.278	0.316	0.376	0.412
Al	0.029	0.801	0.978	4.298	3.083	0.18	0.386	0.553	0.495	0.872	0.684	0.219
Si	0.522	0.633	0.421	1.907	0.897	0.048	0.799	0.36	0.04	1.887	0.841	0.038

are altered to some extent against changes in soil water content by the precipitated, exchangeable, or adsorbed zones in the solid phase of soil (Wolt, 1994). Increases in soil water content resulted in changes of ionic concentrations, distribution, and complex structure formation (Fotovat and Naidu, 1998). Karst soils are rich in Ca and Mg and their concentrations are interactive.

Soil Ca is typically maintained by increasing precipitation and calcite dissolution. The dissolution of CaCO_3 in high moisture soils is a key factor in the dissolving of HCO_3^- , which is evident from the decline in soil Ca (Misra, 2003; Misra and Tyler, 1999). From this

perspective, low Zn in the soil solution with increasing soil water content might be a consequence of high HCO_3^- , which resulted in a decrease in Zn availability due to increased precipitation (Sajwan and Lindsay, 1986). The decreased concentrations of rock-derived nutrients in soils (particularly Ca and Zn) due to increased precipitation, suggested that the effects of increased precipitation on leaching exceeded its impacts on weathering and deposition in wetter sites. The adsorption rate of K was primarily handled by water supplies to the soil (Barber, 1995). Additionally, the availability of K in soil, and the efficient uptake of applied K in

plant species increasing with soil moisture have been well documented in previous studies (Misra and Tyler, 1999; Singh and Singh, 2004; Zeng and Brown, 2000).

4.2. Differential variations of elemental concentrations in shrubs and grasses with increasing precipitation

Based on our results, the non-metal elements (N and P in particular) remained unchanged, while the metal elements exhibited variable patterns in shrubs and grasses. This suggested differences in the physiological functions of these elements, and the biological traits of plants. For instance, the addition of water had significant impacts on foliar composition; a decrease in the concentrations of leaf K, Mg, S, and an increase in C:K, N:K, and P:K ratios in shrubs was noted (Fig. S6). In contrast, the grass species had higher leaf K, Fe, Si, and lower C:K, N:K, and P:K ratios (Fig. S7).

Such variable phenomena have been observed in previous studies (Gherardi and Sala, 2015; Lu et al., 2006). Our results were consistent with the findings that the leaf N and P concentrations of *Cinnamomum burmanni* seedlings were not affected by increasing precipitation (Wang et al., 2019). It was reported that precipitation and N-addition treatments had no significant effect on the N:P ratios of leaves. According to Singh and Singh (2004) higher concentrations of K, Mg, S, Ca, and Zn were found in the shoots of two grass species (*Festuca ovina* and *Agrostis stolonifera*) at 50% moisture levels. Gargallo Garriga et al. (2014) reported that two grass species (*Holcus lanatus* and *Alopecurus pratensis*) contained lower K, Mg, S, and higher C contents with increasing drought during the summer season, while no significant changes were observed for Fe, Mn, and Na. Further, a study reported that a watering treatment significantly increased the concentration of Si in the dominant wild grass species (*Themeda triandra*), but not for the lawn species (*Digitaria macroblephara*) (Quigley and Anderson, 2014).

Such responses of elemental concentrations in grasses and shrubs to increased precipitation might be the result of the combination of multiple factors. Firstly, the differential responses of shrubs and grasses to increased precipitation could be related to competitive interactions and different rooting patterns (Gherardi and Sala, 2015). Being relatively shallow-rooted, grasses use moisture from the upper soil layers (Jackson et al., 1996), whereas deep-rooted shrubs obtain most of their nutrients from deep soil layers (Sala et al., 1992; Weltzin and McPherson, 2000). Thus, differences in the availability of nutrients may initiate a competitive interaction between the two plant types (Weltzin and McPherson, 2000).

Secondly, it might also be due to differences in the soil water content, owing to the distinct growth patterns of the two plant types during an annual cycle. Both the grasses and shrubs in this investigation began with new growth under warming temperatures in the spring season. Owing to differences in water systems (e.g., groundwater versus surface water), shrubs can grow more quickly than grasses during this season under increasing precipitation. This is because the growing points of shrubs are positioned at the branch tips, while grasses grow from their bases in soil systems (Schacht et al., 2005).

However, ideal air temperatures (AT) for all tea varieties can assist with breaking the dormancy of buds. It was found that AT is a good condition to break dormancy and increase development, ultimately toward good yields for tea varieties. During the dry season, the daily AT increased in our research plots, and peaked between 8:00 and 16:00 (Fig. S10). Wild grass species such as *Dactylis glomerata* (Volaire, 2002) and *Poa bulbosa* (Volaire et al., 2001) exhibited summer dormancy in the Poaceae family. These species displayed full dormancy and totally ceased development, as their meristems, even under summer irrigation, were forced into endogenously induced dehydration (Volaire and Norton 2006).

Thirdly, the utilization of macro- and micronutrients is essential for the survival of plant species, each of which is vital for completing their life cycles. The metabolic functions of most nutrients proceed primarily in the leaves, where photosynthesis and protein synthesis are most active (Nunes-Nesi et al., 2014). Multiple elements contribute either to the structures or activities of the various enzymes that are involved in these processes (Tabatabai, 1994). These elements can be categorized in terms of their influence on growth: P, S, and N are major elements for the support of proteins and nucleic acids, while Ca, Mg, and K are linked with plant structures and photosynthetic activities, and Zn and Fe are important for enzymatic activities (Ågren and Weih, 2012). The groupings of these elements in plant species have different biological functions (Maathuis and Diatloff, 2013).

Foliar K is mostly present in cell sap as a dissolved ion (Meena et al., 2016). Potassium plays a significant role in the activation of many enzymes that are vital for photosynthesis and respiration, as an osmotic regulator, and for stomatal control (Kalaji et al., 2016). Although Si is not generally mentioned as an essential element or nutrient of plants, it does play a significant role in their growth, mineral nutrition, mechanical strength, and responses to environmental stress (Epstein, 1994). As observed in our study, grasses take up more K, Fe, and Si due to their increased soil water content. The grasses reacted more readily than shrubs in terms of K uptake, particularly at high soil water content and fertilization levels (Gómez-Carabali et al., 2010).

Species belonging to the Poaceae family accumulate Si in excess amounts, and are known as Si accumulators (higher than 1% of dry matter), while most dicotyledonous plants contain less than 1% Si of dry weight (Sacala, 2009). Quigley and Anderson (2014) observed the effects of watering treatments on the leaf Si content of grasses. They reported that watering treatments strongly influenced the leaf content of Si in grass species, which involved a pattern of increasing silica content in tall wild grass (i.e., *Themeda*). This accumulation of Si may result in improved K⁺ supplies and the uptake of mineral ions, which involves the activity of H-ATPase enzymes. This membrane-bound enzyme facilitates the motility of protons (H⁺) that are employed to transport ions within cells (Kaya et al., 2006).

Finally, the differential responses of the two plant types to increased precipitation might be associated with different stress factors, such as elemental concentrations in soils and soil water content. Numerous K-regulation strategies have been found for K-transporters, which are triggered by different stress factors, such as soil water content and soil K⁺, Ca²⁺, or Na⁺ concentrations (Pyo et al., 2010; Very and Sentenac, 2003; Zhang et al., 2006). High-affinity transport systems (HATS) and low-affinity transport systems (LATS) have been identified in plant cells (Fig. 6). Briefly, LATS are considered to be channel-mediated, function at high concentrations of outer K⁺, and are thermodynamically passive (Szczerba et al., 2005, 2009). In contrast, HATS are saturable transport systems that thermodynamically catalyze the active uptake of K⁺ at low concentrations of outer K⁺ (<1 mM) (Kochian et al., 1989; Szczerba et al., 2009). HATS, as opposed to LATS, are up-regulated at lower concentrations of external K⁺ and down-regulated at high concentrations of external K⁺ (Maathuis and Sanders, 1994; Maathuis et al., 1997). Hence, different plant types can adopt differential K-regulation strategies for dealing with variable water systems due to increased precipitation.

4.3. Contrasting patterns of leaf K stoichiometry in shrubs and grasses with water addition

It is well recognized that among elements, K is strongly involved with the plant water relationship, through osmotic control and the improvement of stomatal function (Babita et al., 2010;

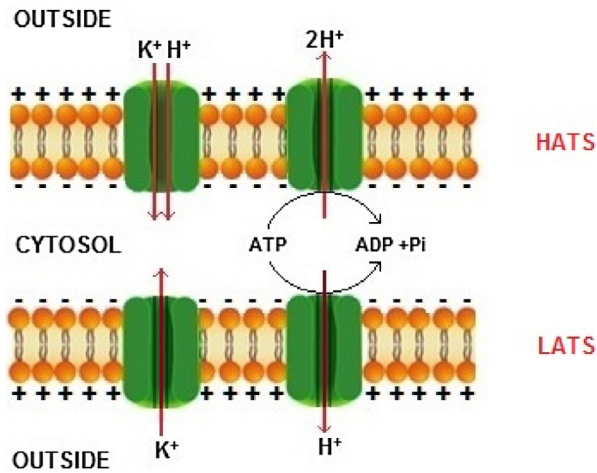


Fig. 6. General mechanisms proposed for K^+ influx into plant cells, via the LATS (low-affinity transport system) and the HATS (high-affinity transport system). In the LATS mechanism, an electrogenic uniport of K^+ is electrically balanced by the ATP-driven efflux of one H^+ . By contrast, in the HATS mechanism, the thermodynamically uphill flux of K^+ is driven by the downhill flux of H^+ ; charge balance is achieved by the outward pumping of two H^+ by the plasma membrane proton ATPase.

Farhad et al., 2011; Sangakkara et al., 2000). Most studies have indicated that lower water supplies caused increases in the concentrations of foliar K in plant species (Gargallo Garriga et al., 2015; Rivas-Ubach et al., 2012, 2014). Furthermore, plant species under dry conditions have a higher capacity to alter their inner K distributions compared to species under wet conditions. Logistically, plants with increased water supplies would contain lower leaf K concentrations.

However, our results clearly revealed that there were contrasting patterns between leaf K concentrations in shrubs (higher) and grasses (lower) with increasing water supplies, which resulted in higher C:K, N:K, and P:K ratios in the former, and lower C:K, N:K, and P:K ratios in the latter (Figs. S6 and S7). With increasing drought, metabolomic changes in the leaves of *Erica multiflora* (shrub) were associated with increased K content, which resulted in lower C:K and N:K, and P:K ratios (Rivas-Ubach et al., 2012). The same trend was observed for shrubs in our study. Recent ecological-stoichiometric studies of natural ecosystems showed that K was associated with the stoichiometric responses of plants to environmental stresses, rather than N and P (Gargallo Garriga et al., 2014; Rivas-Ubach et al., 2014; Sardans and Peñuelas, 2015). In fact, such variations in foliar K stoichiometry in response to increasing soil moisture was a result of leaf K, due to stable leaf C, N, and P concentrations in plants.

Due to the rapid infiltration of rainfall into belowground systems, grass species in drier surface soils accumulate higher K concentrations in their leaf tissues, which results in lower C:K, N:K, and P:K ratios. The higher accumulation of foliar K may assist with increasing stress-related metabolomic compounds (Rivas-Ubach et al., 2012). Consistent with previous results, we found in one of our recent studies that higher precipitation levels caused a significant increase in the content of leaf metabolites in two grass species (*A. setosa* and *C. distans*) and a decrease in the content of leaf metabolites in two shrub species (*B. brachycarpa* and *C. spinarum*) (Umair et al., 2019). Certainly, the leaf K concentrations co-varied with the overall metabolome, which indicated the significance of foliar K in plant functions, despite its complete absence in biomolecules (Rivas-Ubach et al., 2012, 2014). Further, K and its elemental ratios C:K, N:K, or K:P may vary with metabolomic shifts in response to environmental changes.

The high permeability of karst surface soil enables the rapid infiltration of rainfall into subterranean systems, where calcite dissolution forms cavities (Bosellini, 1989). According to Ries et al. (2015), soil water content data showed the rapid infiltration of water into the deeper portions of soil profiles during strong rainfall events in a semi-arid karst region. Thus, the deeper soil layers possessed increased moisture content due to higher precipitation. Grasses with shallow roots can only access water located in the surface layers of the soil.

Consequently, grass species increase foliar K concentrations and adopt K-regulation strategies to maintain the water potential in drier surface soils during the growing season. When grasses and shrubs are grown together, the grasses compete more successfully for soil K and the shrubs are more liable to become deficient, which is likely due to differences in rooting pattern (Hopper and Clement, 1967). This mechanism can explain why grasses take up more K in cases of higher precipitation, whereas shrubs have lower K concentrations.

4.4. Associations of elemental concentrations in leaves with element availability in soils

Our findings clearly showed that the addition of water did not alter the N and P concentrations in the leaves of four plant species (Table 2). With increasing soil moisture from CK to T3, leaf N and P remained unchanged and showed stability with soil supply during the dry season (Table 2). Likewise, Wang et al. (2019) found that a two-year watering regime had no significant impact on the concentrations of N and P in the soils and leaves of plant species. Previous investigations also revealed that macronutrients were more strictly regulated as compared to microelements (Gojon et al., 2009; Karimi and Folt, 2006).

Our results confirmed the hypothesis that macroelements (i.e., N and P) are strictly regulated in contrast to other elements (Table 2). Karimi and Folt (2006) reported that organisms strictly regulated non-metal elements (C, N, and P) and weakly regulated non-essential metals in a natural ecosystem. Because of the extensive limitations of N and P in natural ecosystems (Elser et al., 2007; Harpole et al., 2011), their concentrations in plants may be increased by enhancing the supply of N and P in soils (Tian et al., 2019; Xia and Wan, 2008; Yuan and Chen, 2015). It was shown that non-metal elements (particularly N and P) in plant species exhibited stability in association with soil supply.

Plants require more N and P for plant growth than any other nutrient (Güsewell, 2004; Koerselman and Meuleman, 1996); however, only a small portion of the N and P in soil is available to plants (Schachtman et al., 1998; Singh and Singh, 1994). These elements are often present in unavailable forms, or in forms that are only available outside the rhizosphere (Schachtman et al., 1998). This may have been a reason for the low correlation between total soil N and P and foliar N and P content in our study.

It has been assumed that foliar N and P may be more closely related to the availability of soil N and P fractions to plants (Ilg et al., 2009; Mader and Thompson, 1969). However, the concentrations of available fractions typically increase with the total nutrient fractions (Black, 1993). Nevertheless, in forests, unlike in many agricultural systems with annual crops, it has been difficult to identify a single measure or fraction of available soil nutrients that can sufficiently predict the nutritional status of plants (Mahendrappa et al., 1986; Niederberger et al., 2019).

Reasonably, macroelements (N, P, and S) are major elements required by proteins and nucleic acids; therefore, they need to be strictly regulated to maintain a relatively constant nutrient composition. In fact, the strict regulation of leaf elements is critical for balancing nutritional demands and maintaining species-specific elemental compositions (Giordano, 2013; Ji et al., 2017). In this

context, Zhou et al. (2015) explained that the degree of homeostatic regulation of elements is more dependent on the nutrient demands of plant species, and nutrient limitations in the surrounding area. The acclimation or adaptation strategies (non-regulatory responses) of organisms may be supported to survive under long-term stressed environments (Giordano, 2013). Because, climate change drivers (e.g., temperature, precipitation, and CO₂ concentrations) may directly impact both the availability of soil nutrients and plant nutrient demands for various metabolic functions, while indirectly influencing the coupling of plant nutrients (Tian et al., 2019).

In our study, foliar K demonstrated plasticity with soil supply during the growing season (Table 2). Similarly, Sardans et al. (2011) reported that foliar K was found to be more variable between dry plant ecotypes than P and N. Furthermore, variable soil nutrients can directly impact plant nutrient uptake and thus alter stoichiometric ratios. Therefore, the plasticity of certain elements, particularly K, might be important for plant species in adapting to nutrient variability with increased precipitation in degraded karst ecosystems.

5. Conclusions

Degraded plant communities in karst areas generally consist of drought-resistant shrubs and grasses with sensitivity to variations in water conditions. Our results revealed that non-metal elements (C, H, N, S, and P, except for Si) in the soil were relatively constant with water addition, whereas metal elements (Fe, K, Mg, Na, and Al) had a significant positive correlation with soil moisture, while Ca and Zn exhibited a negative correlation.

This suggested the strong effects of increasing rainfall on almost all metal elements in soils during the spring period. With the addition of water, leaf N and P remained unchanged in all four species and showed stability with soil supply, while leaf K, Mg, and S decreased in both shrubs (higher C:K, N:K, and P:K), and leaf Fe, Si, and K increased in both grasses (lower C:K, N:K, and P:K).

Due to the rapid infiltration of rainfall into subterranean systems, grass species in drier surface soils accumulated higher K in leaf tissues and adopted a K-regulation strategy to maintain the water potential during the dry season. These results suggested that increasing precipitation in the future might differentially impact the stoichiometry and dynamics of multiple elements in both soils and plants in the degraded karst areas of Southwest China.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.134421>.

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