Effects of high Ca and Mg stress on plants water use efficiency in a Karst ecosystem

Rui Qu and Guilin Han
Institute of Earth Sciences, China University of Geosciences (Beijing), Beijing, China

ABSTRACT

Background: Karst ecosystems are widely distributed in the world, with one of the largest continuous Karst landforms in Southwest China. Karst regions are characterized by water shortage, high soil calcium (Ca) and magnesium (Mg) content, and soil nutrient leaching, resulting in drought stress and growth limitation of plants.

Methods: This study compared nitrogen (N), phosphorus (P), potassium (K), Ca, and Mg of herbaceous and woody plants in a small Karst ecosystem in Southwest China. The indexes of water use efficiency (WUE) were calculated to identify the drought stress of plants in this Karst ecosystem. Meanwhile, the relationship between Ca and Mg accumulation and WUE was evaluated in herbaceous and woody plants.

Results: Herbaceous plants showed a higher content of leaf N (13.4 to 40.1 g·kg$^{-1}$), leaf P (2.2 to 4.8 g·kg$^{-1}$) and leaf K (14.6 to 35.5 g·kg$^{-1}$) than woody plants (N: 10.4 g to 22.4 g·kg$^{-1}$; P: 0.4 to 2.3 g·kg$^{-1}$; K: 5.7 to 15.5 g·kg$^{-1}$). Herbaceous plants showed a significantly positive correlation between WUE and K:Ca ratio ($R = 0.79$), while WUE has a strongly positive correlation with K:Mg ratio in woody plants ($R = 0.63$).

Conclusion: Herbaceous plants suffered from nitrogen (N) limitation, and woody plants were constrained by P or N+P content. Herbaceous plants had higher leaf N, P, and K than woody plants, while Ca and Mg showed no significant differences, probably resulting from the Karst environment of high Ca and Mg contents. Under high Karst Ca and Mg stress, herbaceous and woody plants responded differently to Ca and Mg stress, respectively. WUE of herbaceous plants is more sensitive to Ca stress, while that of woody plants is more sensitive to Mg stress. These findings establish a link between plant nutrients and hydraulic processes in a unique Karst ecosystem, further facilitating studies of the nutrient-water cycling system in the ecosystem.

INTRODUCTION

Karst landforms are widespread globally, accounting for 15% of the global land area (Liu et al., 2020b; Zhang et al., 2021). Southwest China possesses one of the largest continuous Karst regions (~550,000 km$^2$) in the world (Han et al., 2021; Wang et al., 2019). Karst ecosystems are characterized by low soil formation, shallow soil layer, high permeability, and water shortage (Green et al., 2019; Liu et al., 2021); thus, plants in Karst regions always suffer from drought stress (Geekiyavanage et al., 2019). Water use efficiency (WUE), defined
by the quantity of water utilized per carbon gain, reflects the plant water strategy under various water circumstances, which is vital for their survival and productivity \((\text{Nie et al., 2014; Yan et al., 2016})\). Leaf δ^{13}C data have been proved to best quantify WUE by reflecting both water conditions and the physiological status of plants \((\text{Cabrera-Bosquet et al., 2007})\), since leaf {^{13}C} discrimination displays a linear correlation with the ratio of intercellular to ambient CO_2 concentration \((\text{Farquhar, Ehleringer & Hubick, 1989})\). Therefore, leaf δ^{13}C data can be a proxy for the WUE of plants.

WUE is influenced by stomatal conductance, photosynthesis, and respiration processes, which are closely related to leaf nutrient concentration \((\text{Cabrera-Bosquet et al., 2007; Si et al., 2015; Zhang et al., 2012})\). Currently, very little is known about the association between WUE and leaf nutrients in the Karst region, though there already exists a few studies on this topic \((\text{Yan et al., 2016; Zhou et al., 2016})\). For example, leaf potassium (K) content affects stomatal conductance, and meanwhile, WUE shows a strong correlation with K content \((\text{Cao et al., 2009; Zhao et al., 2007})\). K is the second most abundant nutrient in leaves after nitrogen (N), essential for plant-water relationships, such as cell osmosis adjustment and stomatal behavior \((\text{Seleiman, 2019; Si et al., 2015; Vodnik et al., 2019})\). However, leaf K shows significantly antagonistic effects with calcium (Ca) and magnesium (Mg) \((\text{Haghshenas, Arshad & Nazarideljou, 2018; Zhou et al., 2013})\). Furthermore, carbonate rocks dominate Karst regions, resulting in Ca and Mg enrichment in soil \((\text{Green et al., 2019; Li et al., 2019})\); thus, the influence of high Ca and Mg stress on the WUE of plants is still unclear. Meanwhile, a lack of essential soil nutrients, such as N and phosphorus (P), limits plant growth and increases the drought stress rate in the Karst region \((\text{Carrière et al., 2020; Liu et al., 2021})\). N and P are two major components of proteins related to energy, participating in the structural compositions of plants \((\text{Ågren, 2004; Seleiman et al., 2021b; Yuan & Chen, 2015})\). Furthermore, the leaf N:P ratio can reflect the nutrient limitation of plants \((\text{Jiang et al., 2021; Qiu, Wei & Li, 2013})\). Additionally, plants play an essential role in nutrient cycling and hydraulic processes, and leaf nutrients (N, P, K) are important indicators by which plants reflect their nutrient status and acquisition mechanisms \((\text{Han et al., 2011; Mao et al., 2018})\).

Therefore, this study analyzed herbaceous and woody plants in a typical Karst region of Southwest China. The goals of this study were to (1) understand the nutrient stoichiometry and limitation of herbaceous and woody plants; (2) examine interactions between WUE and plant nutrition, and (3) discuss the effects of high Ca and Mg stress on the WUE of herbaceous and woody plants in the Karst ecosystem, and further provide information for nutrient cycling and hydraulic processes of plants in a unique Karst ecosystem.

**MATERIALS AND METHODS**

**Study area and sample collection**

The study area is located in Puding County, Anshun City, Guizhou Province in Southwest China \((26^\circ 15' \text{ to } 26^\circ 16' \ N, 105^\circ 46' \text{ to } 105^\circ 47' \ E)\) \((\text{Fig. 1})\), a typical Karst area with the dominant dolomite bedrock. Dolomite and limestone from Middle Triassic generated calcareous soil \((\text{Han et al., 2020; Zhao et al., 2010})\) and were further transported and deposited as quaternary soil with high Ca and Mg contents. Subtropical monsoons
dominate the climate of the study area, with an annual mean temperature of 15.1 °C and precipitation of 1,400 mm, respectively.

A total of 19 plant samples, including seven types of herbaceous plants and 12 types of woody plants, were collected in this Karst region in June 2016. The herbaceous plants include peanut (*Arachis hypogaea* Linn.), sunflowers (*Helianthus annuus* L.), bracken (*Pteridium aquilinum*), roof iris (*Iris tectorum* Maxim.), taro (*Colocasia esculenta*), Indian chrysanthemum (*Chrysanthemum indicum*), and horseweed (*Conyza canadensis*). In contrast, the woody plants include Chinese firethorn (*Pyracantha fortuneana*), bird cherry (*Prunus padus* L.), oak tree (*Quercus fabri*), Chinese catalpa (*Catalpa ovata*), wheel wingnut (*Cyclocarya paliurus*), prickly wild rose (*Rosa acicularis*), Japanese camellia (*Camellia japonica*), dahurian buckthorn (*Rhamnus davurica*), litsea cubeba (*Litsea pungens* Hemsl.), lobular buckthorn (*Rhamnus parvifolia* Bunge), Chinese ilex (*Ilex chinensis* Sims), and Eucommia bark (*Eucommia ulmoides* Oliver). The target plants are dominant and relatively abundant in the study area, mainly sampled from the upper canopies and with three to five healthy leaves of similar ages at random of the same plants. The collected leaves were rinsed with deionized water (Cascada™, 18.2 MΩ cm). The tissues were cleaned and then freeze-dried. Before elemental analysis, the dried samples were crushed to a powder and passed through a sieve (<150 μm).
Chemical analysis and calculation of WUE

After grounding into uniformly fine powders, the N contents of leaf samples were determined by an elemental analyzer (Elementar, Langenselbold, Germany) at the Surficial Environment and Hydrological Geochemistry Laboratory of the China University of Geosciences (Beijing). Before analyzing other nutrients, the sample powders were dissolved in 3 mL concentrated HNO₃ for over 48 h inside a high-pressure steel bomb at 190 °C in an oven. The digested samples were then analyzed for P, K, Ca, and Mg contents by Optima 5300DV ICP-OES (PerkinElmer, USA). The N content was measured by a multi-element analyzer (Vario TOC Cube, Elementar, Langenselbold, Germany) (Zeng et al., 2022). The samples were randomly measured again to ensure the recovery percentage from 95% to 105% (Gao et al., 2021; Wang et al., 2021). All plant species and nutrients data were shown in Table S1 in the Supplemental File. The stable carbon isotopic ratio (¹³C/¹²C) was measured using an isotope mass spectrometer (MAT-253;Thermo-Finnigan, San Jose, California, USA) at the Institute of Geochemistry, Chinese Academy of Sciences. The data of δ¹³C were from Liu, Han & Zhang (2020a). The δ¹³C values were described as the following equation:

\[ \delta^{13}C (\text{‰}) = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) / R_{\text{standard}} \right] \times 1,000 \]  

(1)

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the ¹³C/¹²C ratios of the sample and standard of Vienna Pee Dee Belemnite, respectively. The external precision was better than ±0.1‰ after multiple measurements of the standard material. The following formula links Δ¹³C to the ratio of CO₂ in the leaf intercellular space to CO₂ in the atmosphere (Cᵢ/Cₐ):

\[ \Delta^{13}C (\text{‰}) = a + (b - a)(C_i/C_a) \]  

(2)

where \( a \) (4.4‰) represents the discrimination against ¹³CO₂ during CO₂ diffusion via stomata, and \( b \) (27‰) reflects carboxylation discrimination (Farquhar & Richards, 1984; O’Leary, 1981). The atmospheric CO₂ concentration \( C_a \) was calculated as the following equation (Feng, 1998):

\[ C_a = 277.78 + 1.350 \exp(0.01572 \times (t - 1740)) \]  

(3)

where \( t \) represents the sampling year, which is 2016 in this study. The WUE can be calculated by the relationship between Δ¹³C and \( C_a \) using the following equation (Hietz, Wanek & Dünisch, 2005; Osmond, Björkman & Anderson, 1980; Peñuelas, Canadell & Ogaya, 2011):

\[ \text{WUE} = \frac{A}{g_{\text{H₂O}}} = C_a(b - \Delta^{13}C) / 1.6(b - a) \]  

(4)

where \( A \) is the net photosynthesis, 1.6 is the ratio value of leaf conductance to water vapor (gH₂O) and CO₂ (gCO₂) (Hietz, Wanek & Dünisch, 2005; Peñuelas, Canadell & Ogaya, 2011).

Data analysis

One-way ANOVA with the least significant difference (LSD) test was performed to test the significant differences between herbaceous plants and woody plants of N, P, K, Ca, and Mg.
contents. Pearson correlation coefficient was used to identify the correlations between WUE and K, Ca, and Mg content. The coefficient of $R^2$ square and $p$-value determined the equations of best-fit lines. Statistical analyses were conducted by the IBM SPSS Statistics for Windows, Version 27.0 (IBM, Armonk, NY, USA).

**RESULTS**

The leaf nutrients (N, P, K, Ca, Mg) and the WUE of herbaceous and woody plants are presented in Fig. 2. The leaf nutrient contents in herbaceous plants are as follows: N contents from 13.4 to 40.1 g·kg$^{-1}$, P contents from 2.2 to 4.8 g·kg$^{-1}$, K contents from 14.6 to 35.5 g·kg$^{-1}$, Ca contents from 11.3 to 44.3 g·kg$^{-1}$, Mg contents from 2.1 to 8.3 g·kg$^{-1}$, and WUE from 38 to 92 µmol·mol$^{-1}$. In woody plants, the leaf contents are: N from 10.4 to...
22.4 g·kg$^{-1}$, P from to 2.3 g·kg$^{-1}$, K from 5.7 to 15.5 g·kg$^{-1}$, Ca from 6.1 to 38.8 g·kg$^{-1}$, Mg from 1.4 to 4.6 g·kg$^{-1}$, and WUE from 49 to 64 μmol·mol$^{-1}$. Overall, these results indicate significant differences in leaf nutrients between herbaceous plants and woody plants in this study area.

**DISCUSSION**

**Leaf stoichiometry in herbaceous plants and woody plants**

Karst soil is characterized by the scarcity of N and P elements but high Ca and Mg contents (Li et al., 2019). Previous studies reported the soil nutrients of N content (3.1 to 7.3 g·kg$^{-1}$), P content (0.5 to 1.18 g·kg$^{-1}$), K content (27.7 g·kg$^{-1}$), Ca content (3.8 to 34.9 g·kg$^{-1}$), and Mg content (0.9 to 22.6 g·kg$^{-1}$) in Puding County (Kuang et al., 2010; Wang et al., 2018). As shown in Figs.2A–2C, herbaceous plants have higher N, P, and K contents than woody plants. Previous studies have reported higher N, P, K, and Mg contents of herbaceous plants compared to woody plants (Borisade, 2020; Tian et al., 2018), which was attributed to the higher growth and turn-over rates and the rapid decomposition of herbaceous plants (Gilliam, 2007; Güsewell & Koerselman, 2002). Our results corroborated the previous findings that herbaceous plants (short life) contained more N and P contents than woody plants (long life) (Güsewell & Koerselman, 2002; Han et al., 2005). However, there were no significant differences in leaf Ca, Mg content between herbaceous plants and woody plants (Figs. 2D and 2E). This phenomenon was probably attributed to the alkaline soil with the enrichment of high Ca and Mg content in the Karst region (Li et al., 2019; Liu et al., 2021), which limited the growth of plants (Carrière et al., 2020). As shown in Fig. 2F, herbaceous plants exhibited a wider WUE range than woody plants. Woody plants are likely to have deep roots and rely on water from deep soil layers, while herbaceous plants rely on water from shallow soil layers (Nie et al., 2014; Nie et al., 2011; Rose, Graham & Parker, 2003; West et al., 2008). Therefore, under the condition of high soil permeability and water shortage in the Karst region (Liu et al., 2021), woody plants should have more steady water-use strategies under drought conditions (Fig. 2F). In other aspects, the various WUE in plants may be related to K content. A possible explanation is that plants collected osmoticum (K$^+$) in response to water stress to raise cell osmotic potential, attracting water into cells (Si et al., 2015). Therefore, herbaceous and woody plants have significantly distinctive nutritional needs, and their WUE reveals quite different features.

Nutrient leaking from thin, calcareous soils in the Karst region may decrease the remaining capacity of soil to deliver essential ecosystem services, further affecting carbon sequestration and food production (Ma et al., 2018; Song et al., 2017). Therefore, it is necessary to understand the type of nutrient limitation in plants. Previous study has suggested several nutrient limitations (Olde Venterink et al., 2003): N limitation (N:P < 14.5 and N:K < 2.1), P or P+N limitation (N:P > 14.5 and K:P > 3.4), and K or K+N limitation (N:K > 2.1 and K:P < 3.4). As shown in Fig. 3, almost all herbaceous plants and several woody plants exhibited N limitation, while the other woody plants were P or N+P limited. These results were consistent with the findings that N limited grassland growth, P limited secondary forest growth, and N+P limited shrub growth in Southwest China (Zhang et al., 2015). None of the herbaceous plants and woody plants suffer from K
limitation. Therefore, agricultural activities in this Karst area should apply more N fertilizer to crops. However, the limitation of plant nutrients does not rule out the dilution effects due to the specific Karst environment. For example, K, Ca, and Mg contents can influence the NO$_3^-$ and NH$_4^+$ uptake of plants (Chen et al., 2021) and the antagonistic interaction between K and Ca (Haghshenas, Arshad & Nazarideljou, 2018). Further study should consider using the isotope tracing method to reveal the specific sources of elements and determine the influence of elements on each other.

**Water use efficiency in herbaceous plants and woody plants**

Drought stress on plants can greatly affect their growth, yield, and grain quality (Seleiman et al., 2021a). Though the precipitation of 1,400 mm is in the study area, water resources in Karst regions are still limited due to shallow soil and high permeability of carbonate rocks, resulting in plants facing drought stress when growing (Liu et al., 2021). The leaf $\delta^{13}$C (from $-25.3$‰ to $-30.41$‰) of all plants is higher than typical subtropical species with leaf $\delta^{13}$C from $-31.1$‰ to $30.5$‰ (Qu et al., 2001), showing plants suffer more drought effect in this area. Under long-term water shortage, plants will employ several strategies in response to drought stress, such as elevating cell osmotic potential or adjusting leaf stomatal conductance (Seleiman et al., 2021a; Si et al., 2015; Vodnik et al., 2019). K plays a critical role in the above functions since K is primarily delivered passively in plant organs through the xylem flow. Regarding leaf cells, guard cells acquire K$^+$ and have a higher concentration than nearby cells, and K$^+$ outflow causes turgor loss in guard cells (Misra, Reichman & Chen, 2019). Turgor loss in the paired guard cells closes the stomatal gap, which controls water and gases (Melkikh & Sutormina, 2022). Previous studies found a positive relationship between leaf K and WUE (Cao et al., 2009; Zhao et al., 2007), which means high leaf K levels can increase stomatal sensitivity to water stress and lower stomatal conductance (representing higher $\delta^{13}$C) (Zhou et al., 2013). However, no significant

![Figure 3 Diagram of nutrient stoichiometry in herbaceous plants and woody plants according to the type of nutrient limitation from Olde Venterink et al., 2003.](https://doi.org/10.7717/peerj.13925/fig-3)
correlation between leaf K and WUE occurred in the Karst region of this study. On the contrary, WUE showed a strong positive correlation with leaf K:Ca ratio in herbaceous plants (Fig. 4A), implying the effects of Ca on the WUE. Meanwhile, there was a strong correlation between leaf K:Ca ratio and leaf Ca content (Fig. 4B), indicating that Ca content mainly controlled the ratio of K and Ca. Similarly, K:Mg ratio showed a strong correlation with WUE and Mg content in the woody plants (Figs. 4C and 4D), indicating the impact of leaf Mg on WUE and the ratio of K and Mg. Under high calcium stress, a large amount of Ca\(^{2+}\) influx inhibited the outflow of H\(^+\) by reducing the activity of plasma membranous H\(^+\)-ATP-ase, which further affected plant stomata, photosynthesis, and transpiration (Sukhova, Akinchits & Sukhov, 2017). Mg plays an essential role in photosynthesis as the central atom of the chlorophyll molecule (Pokharel et al., 2018). Furthermore, the antagonistic effects of Ca and Mg on K in plants have been widely reported (Haghshenas, Arshad & Nazarideljou, 2018; Zhou et al., 2013). Therefore, WUE was affected by the joint influence of leaf Ca, Mg, and K content under high Ca and Mg stress in the Karst region. Additionally, the WUE of herbaceous plants was more sensitive to Ca, whereas the WUE of woody plants was more sensitive to Mg.
CONCLUSIONS
This study provided leaf nutrients (N, P, K, Ca, and Mg) stoichiometry and water use efficiency of herbaceous plants and woody plants in a typical Karst region. Herbaceous plants showed the characteristics of N limitation, and woody plants exhibited P or N+P limitation in the study area. N, P, K content showed significant variations between herbaceous plants and woody plants, while no significant differences occurred in Ca and Mg content due to high Ca and Mg stress in the Karst region. Under high Ca and Mg stress, WUE displayed a strong positive correlation with K:Ca ratio in herbaceous plants and with K:Mg ratio in woody plants, indicating that herbaceous plants were more sensitive to Ca and woody plants were more sensitive to Mg. Our findings provided information on nutrient-water interaction by revealing relationships between leaf nutrients and hydraulic processes in terrestrial plants under high Ca and Mg stress in a unique Karst region, further facilitating studies of nutrient and hydrological cycles in the ecosystem. Further research is required to investigate the water-use strategies of widespread species responding to environmental conditions and explore the water cycle from root to leaf linked to the nutrients.

ACKNOWLEDGEMENTS
The authors thank Man Liu for field sampling and Qian Zhang for laboratory analysis.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was supported by the National Natural Science Foundation of China (No. 41661144029 and No. 41325010) and the 2021 Graduate Innovation Fund Project of China University of Geosciences, Beijing, (No. ZD2021YC033). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 41661144029 and 41325010.
2021 Graduate Innovation Fund Project of China University of Geosciences, Beijing: ZD2021YC033.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
• Rui Qu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
• Guilin Han conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
Data Availability
The following information was supplied regarding data availability:

The raw data are available in the Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13925#supplemental-information.

REFERENCES

Ågren GI. 2004. The C:N:P stoichiometry of autotrophs–theory and observations. *Ecology Letters* 7(3):185–191 [DOl 10.1111/j.1461-0248.2004.00567.x](http://dx.doi.org/10.1111/j.1461-0248.2004.00567.x).

Borisade TV. 2020. Nutrient status in herbaceous layer of riparian forests in Southwestern, Nigeria. *Tropical Ecology* 61(4):589–593 [DOI 10.1007/s42965-020-00105-6](http://dx.doi.org/10.1007/s42965-020-00105-6).

Cabrera-Bosquet L, Molero G, Bort J, Noguès S, Araus JL. 2007. The combined effect of constant water deficit and nitrogen supply on WUE, NUE and Δ13C in durum wheat potted plants. *Annals of Applied Biology* 151(3):277–289 [DOI 10.1111/j.1744-7348.2007.00195.x](http://dx.doi.org/10.1111/j.1744-7348.2007.00195.x).

Cao SK, Feng Q, Si JH, Su YH, Chang ZQ, Xi HY. 2009. Relationships between foliar carbon isotope discrimination with potassium concentration and ash content of the riparian plants in the extreme arid region of China. *Photosynthetica* 47(4):499–509 [DOI 10.1007/s11099-009-0075-7](http://dx.doi.org/10.1007/s11099-009-0075-7).

Carrière SD, Martin-StPaul NK, Cakpo CB, Patris N, Gillon M, Chaliakis K, Doussan C, Olioso A, Babic M, Jouineau A, Simioni G, Davi H. 2020. The role of deep vadose zone water in tree transpiration during drought periods in karst settings – Insights from isotopic tracing and leaf water potential. *Science of the Total Environment* 699:134332 [DOI 10.1016/j.scitotenv.2019.134332](http://dx.doi.org/10.1016/j.scitotenv.2019.134332).

Chen CJ, Wu YJ, Wang SH, Liu ZT, Wang GA. 2021. Relationships between leaf delta N-15 and leaf metallic nutrients. *Rapid Communications in Mass Spectrometry* 35(2):229 [DOI 10.1002/rcm.8970](http://dx.doi.org/10.1002/rcm.8970).

Farquhar G, Ehleringer J, Hubick K. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40(1):503–537 [DOI 10.1146/annurev.pp.40.060189.002443](http://dx.doi.org/10.1146/annurev.pp.40.060189.002443).

Farquhar G, Richards R. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology* 11(6):539–552 [DOI 10.1071/PP9840539](http://dx.doi.org/10.1071/PP9840539).

Feng X. 1998. Long-term c13/c12 response of trees in western North America to atmospheric CO2 concentration derived from carbon isotope chronologies. *Oecologia* 117(1–2):19–25 [DOI 10.1007/s004400506262](http://dx.doi.org/10.1007/s004400506262).

Gao S, Wang Z, Wu Q, Wang W, Peng C, Zeng J, Wang Y. 2021. Urban geochemistry and human-impacted imprint of dissolved trace and rare earth elements in a high-tech industrial city, Suzhou. *Elementa: Science of the Anthropocene* 9(1):1 [DOI 10.1525/elementa.2020.00151](http://dx.doi.org/10.1525/elementa.2020.00151).

Geekiyanaage N, Goodale UM, Cao KF, Kitajima K. 2019. Plant ecology of tropical and subtropical karst ecosystems. *Biota tropica* 51(5):626–640 [DOI 10.1111/btp.12696](http://dx.doi.org/10.1111/btp.12696).

Gilliam FS. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience* 57(10):845–858 [DOI 10.1641/B571007](http://dx.doi.org/10.1641/B571007).

Green SM, Dungait JAJ, Tu CL, Buss HL, Sanderson N, Hawkes SJ, Xing KX, Yue FJ, Hussey VL, Peng J, Johnes P, Barrows T, Hartley IP, Song XW, Jiang ZH, Meersmans J, Zhang XY, Tian J, Wu XC, Liu HY, Song ZL, Evershed R, Gao Y, Quine TA. 2019. Soil
functions and ecosystem services research in the Chinese karst Critical Zone. *Chemical Geology* **527**(3):15 DOI 10.1016/j.chemgeo.2019.03.018.

Güsewell S, Koerselman W. 2002. Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology, Evolution and Systematics* **5**(1):37–61 DOI 10.1078/1433-8319-000022.

Haghshenas M, Arshad M, Nazarideljou MJ. 2018. Different K:Ca ratios affected fruit color and quality of strawberry 'Selva' in soilless system. *Journal of Plant Nutrition* **41**(9):243–252 DOI 10.1080/01904167.2017.1385797.

Han W, Fang J, Guo D, Zhang Y. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* **168**(2):377–385 DOI 10.1111/j.1469-8137.2005.01530.x.

Han WX, Fang JY, Reich PB, Ian Woodward F, Wang ZH. 2011. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecology Letters* **14**(8):788–796 DOI 10.1111/j.1461-0248.2011.01641.x.

Han G, Tang Y, Liu M, Van Zwieten L, Yang X, Yu C, Wang H, Song Z. 2020. Carbon-nitrogen isotope coupling of soil organic matter in a karst region under land use change, Southwest China. *Agriculture Ecosystems & Environment* **301**:107027 DOI 10.1016/j.agee.2020.107027.

Han R, Wang Z, Shen Y, Wu Q, Liu X, Cao C, Gao S, Zhang J. 2021. Anthropogenic Gd in urban river water: a case study in Guiyang, SW China. *Elementa: Science of the Anthropocene* **9**(1):00147 DOI 10.1525/elementa.2020.00147.

Hietz P, Wanek W, Dünisch O. 2005. Long-term trends in cellulose $\delta^{13}$C and water-use efficiency of tropical Cedrela and Swietenia from Brazil. *Tree Physiology* **25**(6):745–752 DOI 10.1093/treephys/25.6.745.

Jiang D, Yang B, Cheng X, Chen HYH, Ruan H, Xu X. 2021. The stoichiometry of leaf nitrogen and phosphorus resorption in plantation forests. *Forest Ecology and Management* **483**:118743 DOI 10.1016/j.foreco.2020.118743.

Kuang Y, Wen D, j Yan, Liu S, Chu G, Zhou C, Wang G, Zhang Q. 2010. Characteristics of element contents in leaves of 3 dominant species in Karst forest in Puding, Guizhou, China. *Chinese Journal of Applied Environmental Biology* **16**(2):158–163 [in Chinese] DOI 10.3724/SP.J.1145.2010.00158.

Li F, He X, Sun Y, Zhang X, Tang X, Li Y, Yi Y. 2019. Distinct endophytes are used by diverse plants for adaptation to karst regions. *Scientific Reports* **9**(1):5246 DOI 10.1038/s41598-019-41802-0.

Liu Q, Deng D, Yao B, Liao Q. 2020b. Analysis of the karst springs’ supply sources in rocky desertification area of Guanling-Huaijiang, Guizhou, China. *Carbonates and Evaporites* **35**(3):90 DOI 10.1007/s13146-020-00623-3.

Liu M, Han G, Zhang Q. 2020a. Effects of agricultural abandonment on soil aggregation, soil organic carbon storage and stabilization: results from observation in a small karst catchment, Southwest China. *Agriculture, Ecosystems & Environment* **288**:106719 DOI 10.1016/j.agee.2019.106719.

Liu C, Huang Y, Wu F, Liu W, Ning Y, Huang Z, Tang S, Liang Y. 2021. Plant adaptability in karst regions. *Journal of Plant Research* **134**(5):889–906 DOI 10.1007/s10265-021-01330-3.

Ma M, Gao Y, Song X, Green SM, Xiong B, Dungait JAJ, Peng T, Quine TA, Wen X, He N. 2018. Migration and leaching characteristics of base cation: indicating environmental effects on soil alkalinity in a karst area. *Environmental Science and Pollution Research* **25**(21):20899–20910 DOI 10.1007/s11356-018-2266-x.
Mao Q, Lu X, Mo H, Gundersen P, Mo J. 2018. Effects of simulated N deposition on foliar nutrient status, N metabolism and photosynthetic capacity of three dominant understory plant species in a mature tropical forest. *Science of the Total Environment* **610-611(6)**:555–562 DOI 10.1016/j.scitotenv.2017.08.087.

Melkhik AV, Sutormina MI. 2022. From leaves to roots: biophysical models of transport of substances in plants. *Progress in Biophysics and Molecular Biology* **169–170(9)**:53–83 DOI 10.1016/j.pbiomolbio.2022.01.002.

Misra BB, Reichman SM, Chen S. 2019. The guard cell ionome: understanding the role of ions in guard cell functions. *Progress in Biophysics and Molecular Biology* **146(10)**:50–62 DOI 10.1016/j.pbiomolbio.2018.11.007.

Nie Y-P, Chen H-S, Wang K-L, Ding Y-L. 2014. Seasonal variations in leaf δ13C values: implications for different water-use strategies among species growing on continuous dolomite outcrops in subtropical China. *Acta Physiologiae Plantarum* **36(10)**:2571–2579 DOI 10.1007/s11738-014-1628-3.

Nie Y-P, Chen H-S, Wang K-L, Tan W, Deng P-Y, Yang J. 2011. Seasonal water use patterns of woody species growing on the continuous dolostone outcrops and nearby thin soils in subtropical China. *Plant and Soil* **341(1–2)**:399–412 DOI 10.1007/s11104-010-0653-2.

O’Leary MH. 1981. Carbon isotope fractionation in plants. *Phytochemistry* **20(4)**:553–567 DOI 10.1016/0031-9422(81)85134-5.

Olde Venterink H, Wassen MJ, Verkroost AWM, De Ruiter PC. 2003. Species richness-productivity patterns differ between N-, P- and K-limited wetlands. *Ecology* **84(8)**:2191–2199 DOI 10.1890/01-0639.

Osmond CB, Björkman O, Anderson DJ. 1980. *Physiological processes in plant ecology*. Berlin, Heidelberg: Springer.

Peñuelas J, Canadell JG, Ogaya R. 2011. Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. *Global Ecology and Biogeography* **20(4)**:597–608 DOI 10.1111/j.1466-8238.2010.00608.x.

Pokharel R, Gerrits R, Schuessler JA, Frings PJ, Sobotka R, Gorbushina AA, von Blanckenburg F. 2018. Magnesium stable isotope fractionation on a cellular level explored by cyanobacteria and black fungi with implications for higher plants. *Environmental Science & Technology* **52(21)**:12216–12224 DOI 10.1021/acs.est.8b02238.

Qiu LJ, Wei XR, Li LH. 2013. Nutrient stoichiometry of three plant species under a natural nutrient gradient of a semiarid small watershed. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* **63(3)**:231–240 DOI 10.1080/09064710.2012.753107.

Qu CM, Han XG, Su B, Huang JH, Jiang GM. 2001. The characteristics of Foliar δ13C values of plants and plant water use efficiency indicated by δ13C values in two fragmented rainforests in Xishuangbanna, Yunnan. *Journal of Integrative Plant Biology* **43**:186–192 (in Chinese).

Rose K, Graham R, Parker D. 2003. Water source utilization by Pinus jeffreyi and Arctostaphylos patula on thin soils over bedrock. *Oecologia* **134(1)**:46–54 DOI 10.1007/s00442-002-1084-4.

Seleiman MF. 2019. Use of plant nutrients in improving abiotic stress tolerance in wheat. In: Hasanuzzaman M, Nahar K, Hossain MA, eds. *Wheat Production in Changing Environments: Responses, Adaptation and Tolerance*. Singapore: Springer Singapore, 481–495.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML. 2021a. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10(2)**:259 DOI 10.3390/plants10020259.
Seleiman MF, Almutairi KF, Alotaibi M, Shami A, Alhammad BA, Battaglia ML. 2021b. Nano-fertilization as an emerging fertilization technique: why can modern agriculture benefit from its use? *Plants* 10(1):2 DOI 10.3390/plants10010002.

Si J, Feng Q, Yu T, Zhao C, Li W. 2015. Variation in populus euphratica foliar carbon isotope composition and osmotic solute for different groundwater depths in an arid region of China. *Environmental Monitoring and Assessment* 187(11):705 DOI 10.1007/s10661-015-4890-y.

Song X, Gao Y, Green SM, Dungait JAJ, Peng T, Quine TA, Xiong B, Wen X, He N. 2017. Nitrogen loss from karst area in China in recent 50 years: an in-situ simulated rainfall experiment’s assessment. *Ecology and Evolution* 7(23):10131–10142 DOI 10.1002/ece3.3502.

Sukhova E, Akinchits E, Sukhov V. 2017. Mathematical models of electrical activity in plants. *The Journal of Membrane Biology* 250(5):407–423 DOI 10.1007/s00232-017-9969-7.

Tian D, Yan ZB, Niklas KJ, Han WX, Kattge J, Reich PB, Luo YK, Chen YH, Tang ZY, Hu HF, Wright IJ, Schmid B, Fang JY. 2018. Global leaf nitrogen and phosphorus stoichiometry and their scaling exponent. *National Science Review* 5(5):728–739 DOI 10.1093/nsr/nwx142.

Vodnik D, Gričar J, Lavrič M, Ferlan M, Hafner P, Eler K. 2019. Anatomical and physiological adjustments of pubescent oak (Quercus pubescens Willd.) from two adjacent sub-Mediterranean ecosites. *Environmental and Experimental Botany* 165(6):208–218 DOI 10.1016/j.envexpbot.2019.06.010.

Wang J, Wen X, Zhang X, Li S, Zhang D-Y. 2018. Co-regulation of photosynthetic capacity by nitrogen, phosphorus and magnesium in a subtropical Karst forest in China. *Scientific Reports* 8(1):7406 DOI 10.1038/s41598-018-25839-1.

Wang T, Wu Q, Wang Z, Dai G, Jia H, Gao S. 2021. Anthropogenic gadolinium accumulation and rare earth element anomalies of river water from the middle reach of Yangtze River Basin, China. *ACS Earth and Space Chemistry* 5(11):3130–3139 DOI 10.1021/acsearthspacechem.1c00238.

Wang K, Zhang C, Chen H, Yue Y, Zhang W, Zhang M, Qi X, Fu Z. 2019. Karst landscapes of China: patterns, ecosystem processes and services. *Landscape Ecology* 34(12):2743–2763 DOI 10.1007/s10980-019-00912-w.

West AG, Hultine KR, Sperry JS, Bush SE, Ehleringer JR. 2008. Transpiration and hydraulic strategies in a piñon-juniper woodland. *Ecological Applications* 18(4):911–927 DOI 10.1890/06-2094.1.

Yan W, Zhong Y, Zheng S, Shangguan Z. 2016. Linking plant leaf nutrients/stoichiometry to water use efficiency on the Loess Plateau in China. *Ecological Engineering* 87:124–131 DOI 10.1016/j.ecoleng.2015.11.034.

Yuan ZY, Chen HYH. 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. *Nature Climate Change* 5(5):465–469 DOI 10.1038/nclimate2549.

Zeng J, Han G, Zhang S, Liang B, Qu R, Liu M, Liu J. 2022. Potentially toxic elements in cascade dams-influenced river originated from Tibetan Plateau. *Environmental Research* 208:112716 DOI 10.1016/j.envres.2022.112716.

Zhang J, Wu Q, Wang Z, Gao S, Jia H, Shen Y. 2021. Distribution, water quality, and health risk assessment of trace elements in three streams during the wet season, Guiyang, Southwest China. *Elementa: Science of the Anthropocene* 9(1):00133 DOI 10.1525/elementa.2021.00133.

Zhang S-B, Zhang J-L, Slik JWF, Cao K-F. 2012. Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecology and Biogeography* 21(8):809–818 DOI 10.1111/j.1466-8238.2011.00729.x.
Zhang W, Zhao J, Pan F, Li D, Chen H, Wang K. 2015. Changes in nitrogen and phosphorus limitation during secondary succession in a karst region in southwest China. *Plant and Soil* 391(1–2):77–91 DOI 10.1007/s11104-015-2406-8.

Zhao L, Xiao H, Liu X, Juan R, Mingfeng L, Maoxian Z. 2007. Correlations of foliar $\Delta$ with K concentration and ash content in sand-fixing plants in the Tengger Desert of China: patterns and implications. *Environmental Geology* 51(6):1049–1056 DOI 10.1007/s00254-006-0374-2.

Zhao M, Zeng C, Liu Z, Wang S. 2010. Effect of different land use/land cover on karst hydrogeochemistry: a paired catchment study of Chenqi and Dengzhanhe, Puding, Guizhou, SW China. *Journal of Hydrology* 388(1–2):121–130 DOI 10.1016/j.jhydrol.2010.04.034.

Zhou YC, Cheng XL, Fan JW, Harris W. 2016. Relationships between foliar carbon isotope composition and elements of C-3 species in grasslands of Inner Mongolia. *China Plant Ecology* 217(7):883–897 DOI 10.1007/s11258-016-0614-9.

Zhou Y-C, Fan J-W, Harris W, Zhong H-P, Zhang W-Y, Cheng X-L. 2013. Relationships between C3 plant foliar carbon isotope composition and element contents of grassland species at high altitudes on the Qinghai-Tibet Plateau, China. *PLOS ONE* 8(4):e60794 DOI 10.1371/journal.pone.0060794.