

Leaf nutrient concentrations associated with phylogeny, leaf habit and soil chemistry in tropical karst seasonal rainforest tree species

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Abstract

Background and aims Leaf nutrient concentrations are predictors of plant growth variation and crucial for biogeochemical cycling. We aimed to explore the effects of phylogeny, leaf habit and soil chemistry on leaf nutrient concentrations in tropical karst environments. **Methods** We sampled top-soils and leaves of co-existing evergreen and deciduous tree species along the continuum of mountain valley, slope and peak in a tropical karst seasonal rainforest. We used phylogenetic comparative methods to determine how leaf nutrient concentrations varied in response to phylogeny, leaf habit and soil chemistry and interacted with each other. **Results** Tree species had large inter- and intra-nutrient variability and were characterized by the combination of P limitation and Ca hyperaccumulation in leaves. The phylogenetic signals in leaf nutrient concentrations were not significant but increased with decreasing evolutionary rates as a result of the best fitted evolutionary process, i.e., stabilizing selection towards an optimum value. Compared with deciduous species, evergreen species had lower nutrient concentration requirements to fulfill

specific biochemical functions in leaves. Along the valley-slope-peak continuum, the correlations between leaf and soil nutrient concentrations were positive for Ca, Mg, P, Cu and Zn and negative for N, S, K and Fe. The strength of interactions differed among leaf nutrients and this largely depended on the divergent biochemical functions among leaf nutrients.

Conclusions Our results suggest that stabilizing selection combined with the biochemical constraints could select the locally adapted evergreen and deciduous species with sufficient phylogenetic variations to produce leaf nutrient concentrations and certain nutrient combinations that should be well-fitted in tropical karst environments.

Keywords Tropical karst seasonal rainforest · Leaf nutrient concentrations · Leaf habit · Soil chemistry · Phylogenetic comparative methods · Stabilizing selection

Introduction

Plant growth is expected to be affected by a variety of macro-nutrients (e.g., N, P, K, Ca, Mg and S) and micro-nutrients (e.g., Fe, Cu and Zn) (Ågren 2008; Kirkby 2012). It's necessary for plants to meet physiological concentration requirements and maintain relatively stable ratios in tissues for healthy growth (Kirkby 2012). Plant species absorb the required nutrients primary from the soil solution and the concentrations of nutrients in plant tissues are thus strongly controlled by soil chemical

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attributes such as nutrient availability. For instance, the correlations between leaf and soil nutrient concentrations are strongly positive for N, P, K, Ca, Mg, S, Na, Mn and Al in national plants (Han et al. 2011) and for N and P in global plants (Reich and Oleksyn 2004). Furthermore, the role of these nutrients and their specific functions in plants are not independent but intrinsically multivariate. For example, the group of nutrients largely constituting the organic material (e.g., C, N and S) forms associations distinct from the group of nutrients esterifying with alcohol groups (e.g., P and B), regulating the osmotic and electrochemical potentials, enzymatic activation and membrane permeability (e.g., K, Ca, Mg and Mn), or chelating the prosthetic groups of enzymes (e.g., Fe, Cu and Zn) in plant tissues (Kirkby 2012; Grusak et al. 2016). Recently, Peñuelas et al. (2008) proposed a biogeochemical niche hypothesis where each species has an optimal nutrient composition and stoichiometry for optimal functioning in a multivariate space generated by the relative proportions of macro- and micro-nutrients in plant tissues. This hypothesis is based on co-existing plant species tending to use macro- and micro-nutrients in optimal proportions as a result of species specialization to particular abiotic and biotic conditions (Peñuelas et al. 2008, 2010; Sardans and Peñuelas 2014). However, the excess or deficient uptake of a nutrient beyond its optimal ratio is often observed in natural plant communities (e.g., leaf P limitation in tropical lowland forests and leaf Ca hyperaccumulation in subtropical karst forests) (Townsend et al. 2010; Liu et al. 2014). Previous studies have indicated that the variability of the excess or deficient uptake of nutrients could lead to restricted plant growth variations (Ågren 2008; Ågren and Weih 2012). It's thus possible that the specific use of various nutrients reflects different strategies of plant growth, resource uptake and even anti-stress mechanisms for maximizing plant fitness within and across environments (Lambers et al. 2010; Sardans et al. 2016). Take species with different leaf habits for example. Evergreen species usually develop thick and long-lived leaves with low concentrations of nutrients (e.g., N, P, K, Ca, Mg, S and Fe), increase the efficient use of nutrients for leaf carbon fixation and slow down the leaf turnover rates of nutrients, especially where nutrient availability is limited (Aerts and Chapin 2000; Elser et al. 2003; Wright et al. 2004; Ågren 2008; Han et al. 2011). In contrast, deciduous species tend to accumulate more leaf nutrients and grow faster than co-existing evergreen species and could replace evergreen species when the increase of nutrient

availability by fertilizer or atmospheric deposition occurs (Aerts and Berendse 1988; Cornelissen et al. 1999; Cornwell et al. 2008).

Because leaf nutrient concentrations often regulate plant growth and the amount of carbon sequestered in terrestrial ecosystems (Ågren and Weih 2012; Townsend et al. 2010), several studies have attempted to explore their evolution (Garten 1978; Thompson et al. 1997; Broadley et al. 2003; Broadley et al. 2004; Kerkhoff et al. 2006; Watanabe et al. 2007; Fyllas et al. 2009; Zhang et al. 2012; White et al. 2012; Metali et al. 2012; Cornwell et al. 2014; Viani et al. 2014; White et al. 2015; Hao et al. 2015; Metali et al. 2015; Sardans et al. 2015, 2016; He et al. 2016; Leal et al. 2017; White et al. 2017; Verboom et al. 2017; Yang et al. 2017; Fernández-Martínez et al. 2017; Neugebauer et al. 2018). The evolution of leaf nutrient concentrations among plant species can be constrained by a series of abiotic and biotic factors. The abundance of different nutrients in leaves tends to decrease as a function of atomic mass, suggesting a chemical constraint (Markert 1987; Watanabe et al. 2007). Plant species tend to exhibit more slow-return leaves (i.e., thicker and long-lived leaves with lower nutrient concentrations) with decreasing temperature and soil nutrient availability, suggesting strong physical (i.e., climatic and edaphic) constraints (Wright et al. 2004; Ordoñez et al. 2009; Han et al. 2011; Jager et al. 2015). Apart from the chemical and physical constraints, accumulating evidence highlights the potentially important role that phylogenetic constraint can play in generating the phenotypic divergence of leaf nutrient concentrations. It's been reported that there are significant phylogenetic signals in leaf concentrations of many nutrients in regional, national and global plants (Peñuelas et al. 2010; Metali et al. 2012; Sardans et al. 2016). For example, leaf P, K, Ca, Mg, Fe, Mn and Al concentrations all show significant phylogenetic signals in tropical and subtropical karst herbaceous species in the Didymocarpoideae subfamily (Hao et al. 2015). Moreover, the significant phylogenetic signals in leaf concentrations of many nutrients have also been detected by partitioning variation at and above family level (e.g., Watanabe et al. 2007; White et al. 2012; Neugebauer et al. 2018). The significant phylogenetic signals in leaf nutrient concentrations suggest the tendency of trait similarity with increasing phylogenetic relatedness (Peñuelas et al. 2010; Hao et al. 2015; Sardans et al. 2016). It's noteworthy that the phylogenetic signals in leaf nutrient concentrations can be

strongly related to evolutionary processes and rates (Revell et al. 2008). The most widely used method for modeling the trait evolution on phylogenetic trees assumes Brownian motion (BM), which can result from random genetic drift (i.e., pure BM; Felsenstein 1973), directional genetic drift (i.e., a trend toward greater or smaller values) (Pagel's directional model; Pagel 1999) and a combination of random genetic drift and stabilizing selection (i.e., a trend towards an optimum value) (Ornstein-Uhlenbeck model, OU model; Lande 1976). There are also many phylogenetic tree transformation models (e.g., Pagel's λ , δ and κ ; Pagel 1999) to fit the trait evolution. It's thus possible to evaluate the accuracy of the chosen evolutionary models by comparing the pure BM model with other models for leaf nutrient concentrations. However, the integrative analyses for the relationships among phylogenetic signal, evolutionary process and rate for leaf nutrient concentrations remain scarce. Several studies suggest that the evolution of many leaf nutrient concentrations (e.g., concentrations of leaf N, P, K, Ca, Mg and Al) could be better explained by OU models than BM models (Metali et al. 2015; Bai et al. 2015; Fernández-Martínez et al. 2017). Under the OU model, there is a central tendency with an attraction strength which could weaken phylogenetic signal but increase evolutionary rate (Butler and King 2004; Ackerly 2009; Bai et al. 2015). In addition, the strong inter-specific relationships between leaf nutrient concentrations suggest that the phylogenetic constraint could also limit their independent evolution. For example, leaf Ca concentration has been found to strongly affect the concentrations of leaf Mg, Fe, Mn and Al in karst plants (Hao et al. 2015; Medina et al. 2017) and concentrations of leaf N and Al in Chinese plants (Zhang et al. 2012). Although the divergent constraints on the evolution of leaf nutrient concentrations have been confirmed, the series of constraints are not mutually independent. Actually, the phylogenetic signals in leaf nutrient concentrations should be the combined results of the long-term evolution under climatic and edaphic conditions and the competitive interactions among species.

Tropical karst ecosystems are characterized by alkaline calcareous soils and the relative dominance of evergreen broad-leaved tree species and provide an ideal scenario to study which factors underlie the ecological and evolutionary dynamics of plant nutrients. Tropical karst seasonal rainforest is considered unique in the world and represents one of the world's most

remarkable types of forest due to the special limestone soils with abundant Ca, relatively high pH and low water storage capacity, the variety of heterogeneous microhabitats and the impacts of monsoon climate (Su and Li 2003; Anderson-Teixeira et al. 2015). Chen (1988) found that there typically exist three soil types along the mountain valley-slope-peak continuum in tropical karst ecosystems, i.e., hydrated brown calcareous soil in valleys, brown calcareous soil in slopes and black calcareous soil at peaks, suggesting the strong spatial heterogeneity in soil chemistry. He also found that the soils are typically shallow and the pH and concentrations of CaCO_3 , organic matter and total N in top-soils usually increase along the mountain valley-slope-peak continuum. Although the edaphic environments in tropical karst ecosystems are extremely fragile, tropical karst seasonal rainforest communities exhibit remarkably high levels of species richness and endemism. For example, Wang et al. (2014) found that there are 223 species along the mountain valley-slope-peak continuum in the permanent Nonggang 15 ha tropical karst seasonal rainforest dynamics plot, suggesting a rich species diversity. They also found that the top 20 individual abundances of tree species are mostly from evergreen species, suggesting the dominance of evergreen species. However, to our knowledge, no study has examined the variations in leaf nutrient concentrations and their integrative associations with phylogeny, leaf habit and soil chemistry, yet such examinations are important for understanding the functional diversity of nutrient strategies, the ecological and evolutionary adaptations to the strong spatial heterogeneity in soils and finally the success of evergreen species in tropical karst forests.

In this study, we measured the concentrations of six macro-nutrients (i.e., N, P, K, Ca, Mg and S) and three micro-nutrients (i.e., Fe, Cu and Zn) in leaves of evergreen and deciduous broad-leaved tree species under different soil conditions in the permanent Nonggang 15 ha tropical karst seasonal rainforest dynamics plot. We then combined a phylogenetic tree of the study species with leaf nutrient concentrations and soil chemistry to address the following questions:

- (1) What are the variations and nutritional statuses in leaf nutrient concentrations across tree species? Based on the negative relationships of leaf nutrient concentrations with nutrient coefficients of variation (CVs) (Han et al. 2011) and atomic masses

(Markert 1987; Watanabe et al. 2007) among plant species, we predicted that the nutrients required in high concentrations in leaves would show the small variations in their concentrations and relatively low values of their atomic masses in tropical karst environments. We tested this prediction by assessing the Pearson's correlations between leaf nutrient concentration and both nutrient CV and atomic mass. Because the combination of leaf P limitation and Ca hyperaccumulation has been found in tropical and subtropical karst plants (Liu et al. 2014; Hao et al. 2015; Medina et al. 2017), we predicted that the combination of P limitation and Ca hyperaccumulation in leaves would hold true for tropical karst seasonal rainforest tree species. To test this prediction, we assessed the nutritional statuses by comparing leaf nutrient concentrations with the physiological concentration requirements (Kirkby 2012), critical leaf concentrations for sufficiency and toxicity (White and Brown 2010) and standard functional leaf ionome of angiosperms (Neugebauer et al. 2018).

- (2) How do leaf nutrient concentrations interact with each other? Considering that leaf nutrients have divergent biochemical functions (Kirkby 2012; Grusak et al. 2016), we expected that the strength of interactions would differ among leaf nutrients in tropical karst environments. Specially, we predicted that leaf Ca concentration would strongly affect the concentrations of many nutrients such as Mg and Fe in tropical karst environments, as found in other karst environments (Hao et al. 2015; Medina et al. 2017). We tested our predictions by using the phylogenetic linear regression analyses to explore the correlations between leaf nutrient concentrations (Tung Ho and Ané 2014).
- (3) What is the strength of phylogenetic signals in leaf nutrient concentrations? Is there variation in phylogenetic signal among leaf nutrient concentrations? What does phylogenetic signal can tell us about trait evolutionary processes and rates? We hypothesized that all the examined leaf nutrient concentrations would show significant phylogenetic signals, as found in tropical and subtropical karst herbaceous species in the Didymocarpoideae subfamily (Hao et al. 2015). According to Revell et al. (2008), the relationship between phylogenetic

signal and evolutionary rate varies depending on the evolutionary model for continuous traits. Furthermore, the concentrations of many leaf nutrients (e.g., N, P, K, Ca and Mg) have different significant levels of phylogenetic signal and vary greatly in evolutionary rate under the BM model in plants growing in nutrient-limited soils (Verboom et al. 2017). We therefore predicted that the strength of phylogenetic signal and evolutionary rate would differ among leaf nutrient concentrations and that the relationship between phylogenetic signal and evolutionary rate would vary depending on the evolutionary model in tropical karst environments. To test our predictions, we first assessed the phylogenetic signals in leaf nutrient concentrations by Pagel's λ and compared the fitting of different evolutionary models (i.e., BM, OU, Pagel's directional and Pagel's δ) to leaf nutrient concentrations (Hernández et al. 2013). We then assessed the relationship between phylogenetic signal and evolutionary rate under different models by Pearson's correlation analysis.

- (4) Do leaf nutrient concentrations differ between evergreen and deciduous species? Considering that deciduous species have generally nutrient-rich leaves in contrast with evergreen species (Han et al. 2011; Liu et al. 2014), we predicted that evergreen species would have lower leaf nutrient concentrations than deciduous species in tropical karst environments. Considering that leaf habit (i.e., evergreen vs deciduous) is a binary discrete variable, we tested this prediction by using the phylogenetic logistic regression analyses to explore the effect of leaf habit on leaf nutrient concentrations (Tung Ho and Ané 2014).
- (5) Do leaf nutrient concentrations vary with the local variations in soil nutrient availability? Based on the general positive correlations between leaf and soil concentrations for mineral nutrients among plant species (Reich and Oleksyn 2004; Han et al. 2011), we predicted that such correlations would also be shown for tree species in tropical karst environments. We tested this prediction by using the phylogenetic linear regression analyses to explore the correlations between leaf and soil concentrations for the examined nutrients (Tung Ho and Ané 2014).

Materials and methods

Site description and species selection

This study was conducted in the permanent Nonggang 15 ha tropical karst seasonal rainforest dynamics plot (22°25'N, 106°57'E), which is situated in the northern margin of the tropical limestone karst region of China and is connected to the global network of forest research plots led by the Center for Tropical Forest Science - Forest Global Earth Observatories (CTFS-ForestGEO) (Anderson-Teixeira et al. 2015). The plot experienced tropical monsoon climate, with averaged 22 °C mean annual temperature and averaged 1350 mm mean annual precipitation. According to the conditions of soil and vegetation distribution, the plot can be classified into three microhabitat types, i.e., mountain peak type at the altitude between 320 m and 370 m, slope type at the altitude between 240 m and 320 m and valley type at the altitude between 180 m and 240 m. In the valley, the soil type is hydrated brown calcareous soil and the dominant evergreen tree species are *Antidesma japonicum* and *Ficus hispida*. In the mountain slope, the soil type is brown calcareous soil and the dominant evergreen tree species are *Sterculia monosperma*, *Cleistanthus sumatranus* and *Excentrodendron hsienmu*. At the mountain peak, the soil type is black calcareous soil and the dominant evergreen tree species are *Diospyros siderophylla* and *Memecylon scutellatum* (Wang et al. 2014). We selected a total of 40 tree species from 17 families and 8 orders including 15 deciduous species and 25 evergreen species along the mountain valley-slope-peak continuum (Fig. 1; supporting information Table S1). The individuals with diameter at breast height (DBH) ≥ 1 cm of the 40 species accounted for 55% of the total individuals with the same DBH scale in the plot, suggesting their major role in forest structure and function (Wang et al. 2014).

Measurements of leaf and soil nutrients

Leaf nutrients were measured on three to ten mature individuals per species in each microhabitat in July in 2013. For each individual, a branch was selected at random from the crown in an attempt to get sun leaves. We harvested the new fully expanded and intact green leaves and sealed them in plastic bags. The harvested leaves were oven-dried at 60 °C to constant weight. Dried leaf samples from each individual plant were

ground to a powder using an electric-mill (FSJ-A05B1, Bear, China). Total N concentration was measured on dry leaf material using the Kjeldahl method, with subsamples of dried plant material digested by K_2SO_4 : $CuSO_4 \cdot 5H_2O$: Se (10:1:0.1) mixture and H_2SO_4 (Bremner 1960). For the determination of total concentrations of other nutrients (P, K, Ca, Mg, S, Fe, Cu and Zn), powder subsamples were digested by concentrated HNO_3 (Zarcinas et al. 1987) in close vessels using a microwave digester (WX-8000, EU Microwave Chemistry Technology Co., Ltd., Shanghai, China). Digested samples were analyzed by an inductive coupled plasma (ICP) emission spectrum (iCAP Qc, Thermo Fisher Scientific, Bremen, Germany). For leaf ICP analyses, an orange leaf standard (Reference GBW10020; Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, Langfang, China) was used as an internal control.

In each microhabitat, we randomly collected soil samples for the upper 10 cm of soil with ten replicates in October 2014. After soil samples were air-dried, we ground them and made them passed through a 2-mm mesh sieve for chemical analysis. We analyzed the concentrations of total N, P, K, Ca, Mg, S, Fe, Cu and Zn in soils using the same methods in leaves. But for soil ICP analyses, a limestone soil standard (Reference GBW07404; Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences, Langfang, China) was used as an internal control.

Construction of the phylogenetic tree

The phylogenetic relationships among the 40 species were inferred from the ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (rbcL) sequences. The rbcL sequences were retrieved in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; supporting information Table S1). We used MEGA 6.0 to align these sequences and infer the phylogenetic tree (Tamura et al. 2013; Hall 2013). Alignment of the total 40 rbcL sequences was performed using ClustalW. The phylogenetic tree was then inferred by the neighbor-joining method based on the Maximum Composite Likelihood (MCL) model, as the use of MCL model could lead to a much higher accuracy of neighbor-joining trees (Tamura et al. 2004). The trees were evaluated using the bootstrap test based on 1000 replicates.

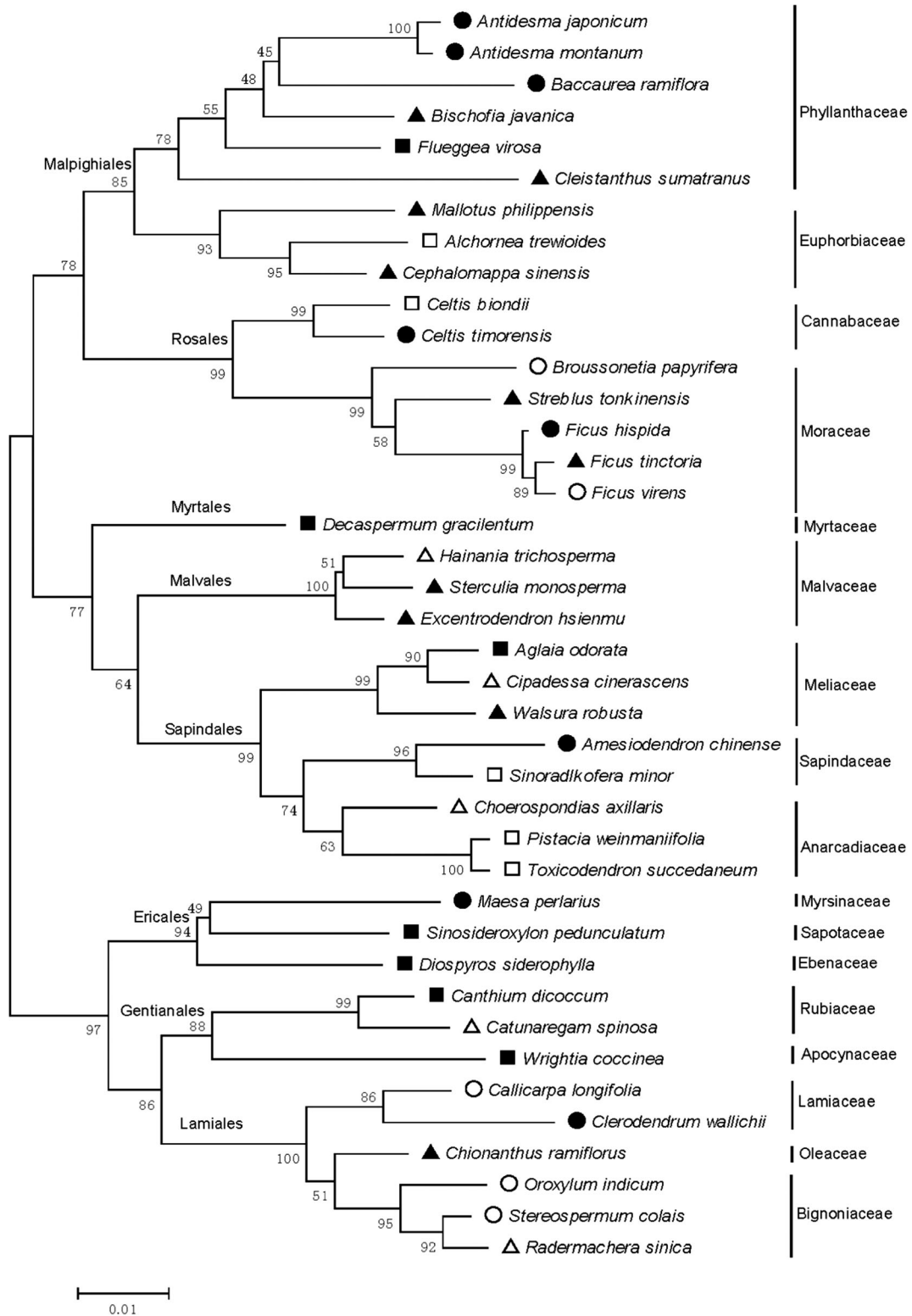


Fig. 1 Phylogenetic tree construction by neighbor-joining method using the rbcL sequences of the evergreen (close symbols) and deciduous species (open symbols) along the continuum of mountain valley (circles), slope (triangles) and peak (squares) in a tropical karst seasonal rainforest. The bootstrap values are shown at branching points. Family divisions are indicated by the broken line on the right side of the tree and order divisions are shown at the clade branches in the tree

The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Finally, we got a non-ultrametric phylogenetic tree of 40 species used for subsequent analyses (Fig. 1).

Estimation of nutritional status

To estimate leaf nutrient concentrations considered to be in the deficient, adequate and toxic ranges, we used the combination of the physiological concentration requirements (Kirkby 2012), critical leaf concentrations for sufficiency and toxicity (White and Brown 2010) and standard functional leaf ionome of angiosperms (Neugebauer et al. 2018). According to Kirkby (2012), the physiological concentration requirements of Ca, N, P, K, Mg, S, Fe, Cu and Zn for adequate plant growth are 2, 15, 2, 10, 5, 1, 0.1, 0.006 and 0.02 mg g⁻¹, respectively. If the concentrations of leaf nutrients are below the physiological requirements, these nutrients are considered to be deficient or limited (e.g., Medina et al. 2017). According to White and Brown (2010), critical leaf concentrations are defined to evaluate the sufficient and toxic ranges in plant species. For example, leaf P concentrations bordering between 2 and 5 mg g⁻¹ and larger than 10 mg g⁻¹ are considered to be in the sufficient and toxic ranges in plant species, respectively. According to Neugebauer et al. (2018), the standard functional leaf ionome of angiosperms derived from a large of sample species grown under nutrient-replete conditions comprises a series of standard leaf concentrations. Specifically, the standard leaf concentrations of Ca, N, P, K, Mg, S, Fe, Cu and Zn are 13.6, 50.4, 7.22, 39.7, 3.61, 3.92, 0.144, 0.0141 and 0.0745 mg g⁻¹, respectively. The standard leaf concentrations lie within the bounds of sufficiency and toxicity for all nutrients (White and Brown 2010; Neugebauer et al. 2018). If the concentrations of leaf nutrients are above the standard leaf concentrations, these nutrients are considered to be highly accumulated. However, it's noteworthy that plant species differ greatly in leaf nutrient concentrations for sufficiency within and across environments (Römheld

2012). For example, the P supply for optimal growth of most plants can be toxic to plants adapted to soils with low P phytoavailability and calcicole and calcifuge species require different Ca supply for optimal growth (White and Broadley 2003; Neugebauer et al. 2018). Therefore, we used the physiological concentration requirements, critical leaf concentrations for sufficiency and toxicity and standard leaf nutrient concentrations without implying that the absolute values hold for all species and all conditions, but considering that the indexes are useful because they provide a relative measure of general nutrient requirements or nutritional statuses for plant growth.

Statistics and phylogenetic comparative methods

We compared the differences in soil nutrient concentrations among microhabitats using the Fisher's least significant difference tests after data log₁₀-transformation. All species mean values of leaf nutrient concentrations were also log₁₀-transformed prior to analysis to achieve normality of the residuals (Kerkhoff and Enquist 2009). We then explored the relationships of leaf nutrient concentrations with nutrient CVs, nutrient atomic masses, physiological concentration requirements and standard leaf nutrient concentrations using Pearson's correlation analysis after data log₁₀-transformation. The above conventionally statistical analyses were conducted in SPSS 13.0 (SPSS, Chicago, IL, USA).

We performed statistical tests in a phylogenetic context using R (version 3.3.3; R Development Core Team 2008). We first conducted the fit of pure BM model plus Pagel's directional model, OU model and Pagel's phylogenetic tree transformation models (i.e., Pagel's λ and δ) to the nutrient variables via the fitContinuous function in the GEIGER package (Harmon et al. 2008). The pure BM model describes how a trait evolves through random-walk corresponding to sigsq, a parameter reflecting the magnitude of evolutionary rate under BM model (Felsenstein 1973; Harmon et al. 2008). The Pagel's directional model describes how a trait evolves towards greater or smaller values over time corresponding to drift, a parameter showing the evolutionary direction and speed of a trait (Pagel 1999; Harmon et al. 2008). The OU model describes how a trait evolves towards an optimum value with a pulling back strength corresponding to α , a parameter describing the strength of selection under OU model. As α increases, the rate of trait evolution along the branches

Table 1 Mean (\pm standard error, SE; mg g^{-1}) and coefficient of variation (CV; %) values of leaf nutrient concentrations and the summary of comparisons of evolutionary model fit to leaf nutrient concentrations in a tropical karst seasonal rainforest

Nutrient	n	Mean \pm SE	BM			Pagel's λ			Pagel's δ			OU		Directional	
			CV	AICc	sigseq	AICc	λ	$P_{\lambda=0}$	$P_{\lambda=1}$	AICc	δ	$P_{\delta=1}$	AICc	α	drift
Ca	40	23.51 \pm 1.01	27.2	-43.92	1.673	-50.09	0.092	0.644	0.004	-49.23	3.009	0.006	-52.01	96.8	-42.13
N	40	20.87 \pm 0.91	27.5	-37.32	1.029	-44.30	0.536	0.101	0.002	-38.97	2.329	0.046	-47.18	89.4	-37.41
P	40	1.216 \pm 0.067	35.1	-12.48	1.915	-19.30	0.507	0.110	0.002	-14.36	2.333	0.040	-21.14	110.7	-12.97
K	40	12.21 \pm 0.84	42.7	12.50	3.576	-3.27	0.049	0.839	<0.001	-1.76	4.687	<0.001	-3.31	142.4	14.73
Mg	40	6.71 \pm 0.54	51.1	27.10	5.152	12.45	0.080	0.674	<0.001	17.21	3.929	<0.001	12.01	189.0	29.22
S	40	2.53 \pm 0.15	37.6	-16.42	1.736	-22.38	0.478	0.212	0.004	-18.97	2.390	0.027	-24.75	84.9	-14.43
Fe	40	0.183 \pm 0.011	38.6	-1.88	2.496	-7.56	0.484	0.089	0.005	-5.71	2.727	0.013	-9.56	64.4	-0.49
Cu	40	0.0076 \pm 0.0007	54.5	33.45	6.039	22.21	0.038	0.873	<0.001	23.98	3.922	<0.001	20.11	180.3	32.96
Zn	40	0.081 \pm 0.008	58.5	32.30	5.867	18.22	0.114	0.593	<0.001	26.51	2.847	0.004	15.30	201.4	31.05

BM describing species traits that have evolved through random-walk. Sigseq is a parameter indicating the trait evolutionary rate under the BM

Pagel's λ describing whether the similarity in species traits is influenced by the phylogenetic relationships of the species (i.e., phylogenetic signal). λ is a parameter indicating the level of phylogenetic signal. $P_{\lambda=0}$ and $P_{\lambda=1}$ indicate the significant level for the observed λ in contrast with values expected under the hypotheses of no phylogenetic signal ($\lambda = 0$) and BM ($\lambda = 1$), respectively

Pagel's δ describing the degree of acceleration or slowness of species trait evolution through the phylogeny. δ is a parameter indicating the trait evolutionary rate under the Pagel's δ . $P_{\delta=1}$ indicates the significant level for the observed δ in contrast with values expected under the hypothesis of constant evolutionary rate ($\delta = 1$)

OU describing trait trend towards an optimum value through the phylogeny. α is a parameter indicating the trait evolutionary rate under the OU

Directional describing trait trend towards greater or smaller values through the phylogeny. Drift is a parameter indicating the evolutionary direction and speed of a trait

BM Brownian motion model; AICc Sample-size corrected Akaike information criterion; OU Ornstein-Uhlenbeck model; and Directional Pagel's directional model

of the tree will be increasing faster, as compared to a pure Brownian process. It's noteworthy that we fitted the OU model with a single optimum instead of more complex models with multiple optima because we had no a priori independent means of estimating potentially different selective regimes for each nutrient. Moreover, the OU model with a single optimum is the simplest and widely used mathematical expression for an evolutionary model that incorporates selection (Butler and King 2004; Gonzalez-Voyer and Kolm 2011; Hernández et al. 2013). Both Pagel's λ and δ models describe how trait evolution is dependent on tree transformation (Pagel 1999). We evaluated the extent to which the phylogeny correctly predicted patterns of similarity in leaf nutrient concentrations (i.e., phylogenetic signal) using the phylogeny scaling parameter, λ (Pagel 1999). We tested whether λ was significantly different from zero (i.e., phylogenetic independence; patterns of trait similarity among species are independent on phylogeny) or unity (i.e., pure BM model; phylogenetic relationship predicts effectively the patterns of similarity between species traits) via the *pgls* function in the CAPER package (Orme et al. 2012). We evaluated whether trait evolution had accelerated or slowed down through the phylogeny using the path-length scaling parameter, δ (Pagel 1999; Hernández et al. 2013). In this test, δ is a parameter describing the differential rates of evolution over time. We used the *pgls* function to test whether δ was significantly different from unity (i.e., the rate of evolution is constant). The values of δ less than unity indicate temporally early trait evolution or "early-burst", indicative of adaptive radiation. The values of δ larger than unity indicate temporally latter trait evolution, indicative of species-specific adaptation. By the way, Pagel's δ model could provide similar evolutionary information to Pagel's κ that is commonly used to fit discrete datasets and is strongly dependent on species extinction or incomplete species sampling (Pagel 1994; Harmon et al. 2008), we therefore didn't showed the κ values of leaf nutrient concentrations. The above fitted models were compared with the sample-size corrected Akaike information criterion (AICc). The model with the lowest AICc value was considered as the best fitted model (e.g., Fernández-Martínez et al. 2017).

Because the OU model had the lowest AICc values for all leaf nutrients (Table 1), we then conducted a series of phylogenetic regression analyses under OU models (e.g., Metali et al. 2015). We used the phylogenetic logistic regression analyses to determine the effect

of leaf habit on leaf nutrient concentrations via the *phyloglm* function in the PHYLOLM package (Tung Ho and Ané 2014). In the running of *phyloglm* function, we set leaf habit as binary response variable (i.e., coding evergreen with "0" and deciduous with "1"), selected the method of logistic-IG10 to optimize the penalized likelihood of the logistic regression and made α for phylogenetic correlation. We used phylogenetic linear regression analyses to explore the strength of interactions among leaf nutrient concentrations via the *phylolm* function in the PHYLOLM package (Tung Ho and Ané 2014). We paid special attention to whether leaf Ca concentration could be as a good predictor for the remaining leaf nutrient concentrations. We also ran the *phylolm* function to explore whether soil nutrient concentrations could well explain leaf nutrient concentrations. In the running of *phylolm* function, we selected the model of OUrandomRoot (i.e., the OU model with the ancestral state at the root having the stationary distribution) for the covariance matrix.

We finally performed Pearson's correlation analysis among \log_{10} -transformed λ , *sigsq*, α , δ and absolute drift to reveal the relationships among phylogenetic signal, evolutionary process and rate in SPSS 13.0 (SPSS, Chicago, IL, USA).

Results

The mean concentrations of the nine nutrients in leaves varied greatly, ranging from 0.0076 mg g⁻¹ for Cu to 23.51 mg g⁻¹ for Ca (Table 1). Mean leaf P concentration (1.216 mg g⁻¹) was lower than the physiological concentration requirement (Supporting information Table S2). Mean leaf N, K, S and Cu concentrations boarded the sufficient leaf concentrations or were below the standard concentrations (Supporting information Table S2). Mean leaf Ca, Mg, Fe and Zn concentrations were higher than the standard leaf concentrations but didn't reach to the toxic leaf concentrations (Supporting information Table S2). The relative variability of the nine nutrients (indicated by their CVs) increased in the order Ca (27.2%) < N (27.5%) < P (35.1%) < S (37.6%) < Fe (38.6%) < K (43.5%) < Mg (51.1%) < Cu (54.5%) < Zn (58.5%) (Table 1). The concentration of different nutrients in leaves decreased significantly as a function of atomic mass ($r = -0.75$, $P < 0.05$) and CV ($r = -0.67$, $P < 0.05$) but increased significantly as a function of physiological requirement ($r = 0.97$, $P < 0.001$) and

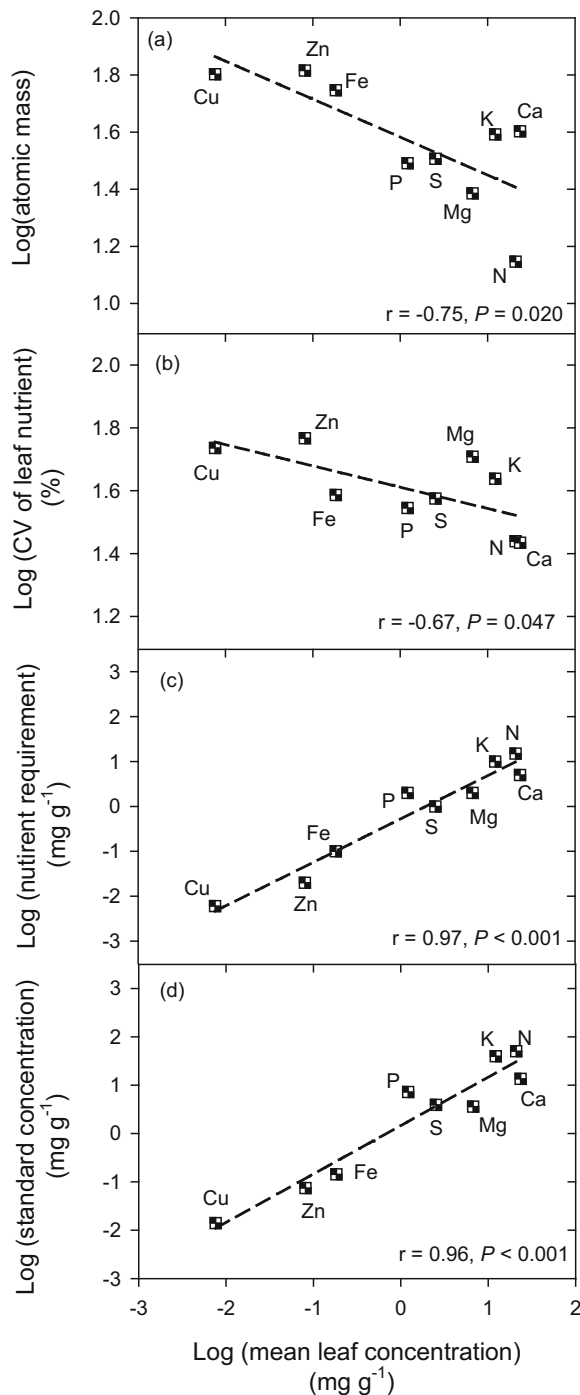


Fig. 2 Leaf concentration of different nutrients as a function of (a) atomic mass, (b) coefficient of variation (CV), (c) physiological nutrient requirement and (d) standard leaf concentration in a tropical karst seasonal rainforest. The coefficients and significant values from Pearson's correlation analyses are shown

standard leaf concentration ($r = 0.96$, $P < 0.001$) (Fig. 2).

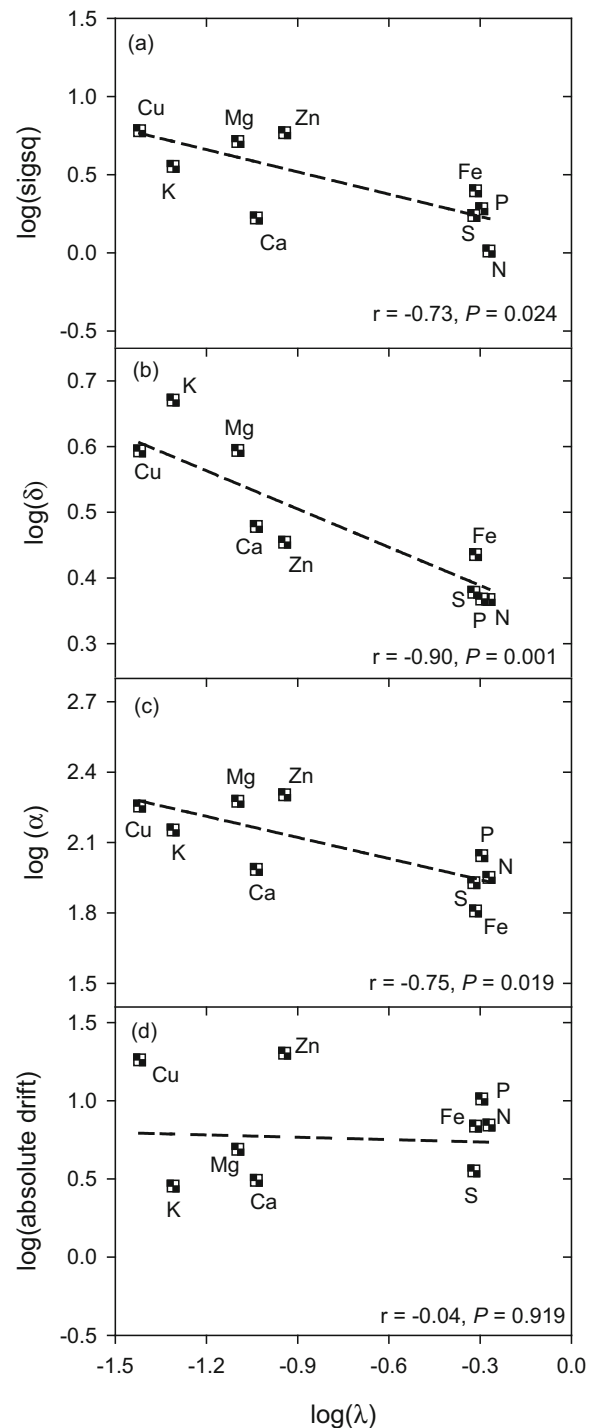


Fig. 3 Phylogenetic signal (λ) as a function of evolutionary rate under (a) Brownian-motion model (sigsq), (b) Pagel's δ model (δ), (c) Ornstein-Uhlenbeck model (α) and (d) Pagel's directional model (absolute drift) across leaf nutrient concentrations in a tropical karst seasonal rainforest. The coefficients and significant values from Pearson's correlation analyses are shown

The values of phylogenetic signal (i.e., λ) were low in leaf Cu, K, Mg and Ca concentrations ($\lambda < 0.15$) and high in leaf N, P, S and Fe concentrations ($\lambda > 0.4$) (Table 1). However, all the λ values were significantly lower than unity but not significantly higher than zero, suggesting the non-significant phylogenetic signals in leaf nutrient concentrations (Table 1). The evolution of leaf nutrient concentrations was best fitted by OU model, as shown by the general lowest AICc values under OU model (Table 1). The Pagel's δ model also fitted the leaf nutrient evolution better than BM model due to the lower AICc values in the former (Table 1). The Pagel's directional model didn't show fitting advantage over BM model due to their general small AICc differences (Table 1).

The parameter representing evolutionary rate under the BM model (i.e., sigsq), Pagel's δ model (i.e., δ), OU model (i.e., α) and Pagel's directional model (i.e., drift) varied greatly among leaf nutrient concentrations (Table 1). For example, the sigsq values ranged from 1.029 (leaf N concentration) to 6.039 (leaf Cu concentration) and the α values ranged from 64.4 (leaf Fe concentration) to 201.4 (leaf Zn concentration) (Table 1). Across the nine leaf nutrients, sigsq, α and δ were strongly and negatively correlated with λ , which in turn had weak negative relationship with absolute drift (Fig. 3).

The presence of leaf habit was strongly associated with the variation in leaf nutrient concentrations (Table 2). Compared with deciduous species, evergreen species had lower leaf Ca, N, K, Mg and Cu concentrations at significant level ($P < 0.05$) and lower leaf P, S,

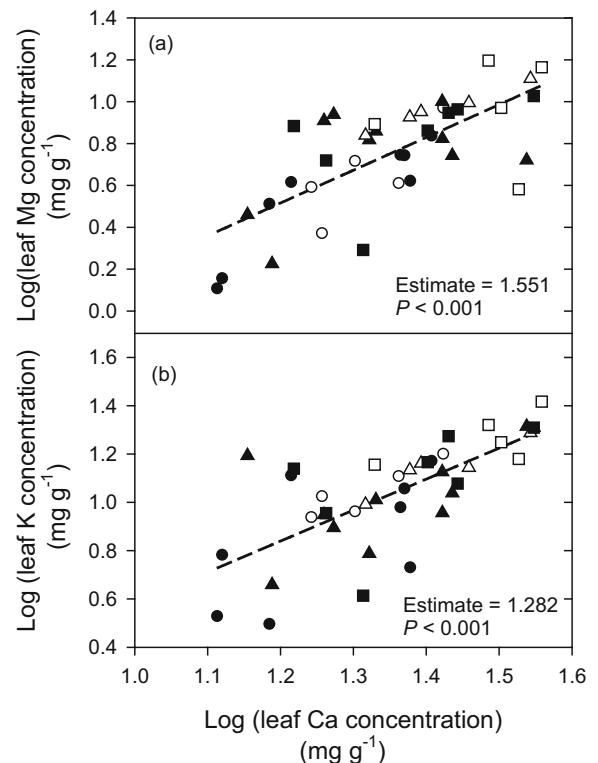


Fig. 4 Leaf Ca concentration as a function of (a) leaf Mg concentration and (b) leaf K concentration across the evergreen (close symbols) and deciduous species (open symbols) along the continuum of mountain valley (circles), slope (triangles) and peak (squares) in a tropical karst seasonal rainforest. The slope estimates and significant values from phylogenetic linear regression analyses are shown

Fe and Zn concentrations at marginal significant level ($P < 0.075$) (Table 2).

Table 2 Summary of phylogenetic logistic regression analyses accounting for variation in leaf nutrient concentrations associated with leaf habit as a binary response variable in a tropical karst seasonal rainforest

Nutrient	Evergreen ($n = 25$)	Deciduous ($n = 15$)	Estimate	P
Ca	21.94 ± 1.22	26.13 ± 1.60	6.32	0.047
N	18.88 ± 1.05	24.19 ± 1.30	10.05	0.009
P	1.117 ± 0.083	1.382 ± 0.106	4.59	0.063
K	10.65 ± 1.02	14.82 ± 1.23	5.18	0.022
Mg	5.82 ± 0.55	8.20 ± 1.04	2.38	0.011
S	2.31 ± 0.17	2.89 ± 0.26	4.08	0.072
Fe	0.165 ± 0.013	0.212 ± 0.019	3.43	0.051
Cu	0.0063 ± 0.0007	0.0097 ± 0.0011	4.31	0.023
Zn	0.072 ± 0.009	0.096 ± 0.013	2.15	0.054

Mean (\pm standard error; mg g^{-1}) leaf nutrient concentrations in evergreen and deciduous species and the statistical slope estimates and P -values are shown

Leaf Ca concentration was significantly and positively correlated with leaf Mg and K concentrations (Fig. 4) but weakly correlated with leaf N, P, S, Fe, Cu and Zn concentrations, which showed significant and positive correlations with each other (Table 3).

Along the valley-slope-peak continuum, the concentrations of soil Ca, N, Mg, S and Fe were highest at peak and the concentrations of soil P, K, Cu and Zn were highest in valley (Table 4). The correlations between leaf and soil nutrient concentrations were significantly positive for Ca, Mg, P, Cu and Zn and significantly negative for N, K, S and Fe (Fig. 5).

Discussion

Variations and nutritional statuses in leaf nutrient concentrations in tropical karst tree species

Leaf nutrient concentrations showed large variations in the tropical karst seasonal rainforest. On one hand, we found over 3000-fold difference between the most (Ca) and least (Cu) abundant nutrient (Table 1), suggesting the large inter-nutrient variability. Consistent with our prediction and other observations (Markert 1987; Watanabe et al. 2007), we found that the concentration of different nutrients in leaves decreased with atomic mass (Fig. 2). The increase of nutrient abundance with decreasing atomic mass could be explained as follows. For nutrients with high atomic masses, the pool sizes of these nutrients could be limited because they tend to be rare in the earth's crust, especially for lanthanide

Table 4 Mean (\pm standard error; mg g^{-1}) soil nutrient concentrations in different microhabitats in a tropical karst seasonal rainforest

Nutrient	Valley ($n = 10$)	Slope ($n = 10$)	Peak ($n = 10$)
Ca	$7.10 \pm 0.92\text{c}$	$10.01 \pm 1.71\text{b}$	$22.10 \pm 1.49\text{a}$
N	$5.25 \pm 0.38\text{c}$	$6.85 \pm 0.43\text{b}$	$9.25 \pm 0.58\text{a}$
P	$2.40 \pm 0.19\text{a}$	$1.29 \pm 0.18\text{b}$	$0.70 \pm 0.04\text{c}$
K	$10.41 \pm 0.31\text{a}$	$8.32 \pm 0.58\text{b}$	$6.27 \pm 0.14\text{c}$
Mg	$3.17 \pm 0.17\text{c}$	$4.27 \pm 0.34\text{b}$	$6.49 \pm 0.28\text{a}$
S	$3.31 \pm 0.23\text{bc}$	$3.93 \pm 0.24\text{b}$	$4.82 \pm 0.19\text{a}$
Fe	$7.32 \pm 0.38\text{b}$	$7.87 \pm 0.37\text{ab}$	$8.56 \pm 0.44\text{a}$
Cu	$0.116 \pm 0.003\text{a}$	$0.090 \pm 0.007\text{b}$	$0.061 \pm 0.002\text{c}$
Zn	$0.654 \pm 0.024\text{a}$	$0.527 \pm 0.034\text{b}$	$0.406 \pm 0.016\text{c}$

The different letters denote significant differences among microhabitats using the Fisher's least significant difference tests ($P < 0.05$)

nutrients (Markert 1987). The trend of low solubility and mobility in water for high atomic mass nutrients would also lead to the low concentrations in plant tissues (McBride et al. 1997). We further found that the concentration of different nutrients in leaves tended to be higher for nutrients with higher physiological requirements (Fig. 2), which is consistent with our prediction and the observations on the Chinese plants reported by Han et al. (2011). This is not surprising because growth would be suboptimal with concentrations below the physiological requirements (Han et al. 2011; Römheld 2012). We also found that the concentration of different nutrients in leaves tended to be higher for nutrients with higher standard concentrations (Fig. 2). According to Neugebauer et al. (2018), standard leaf nutrient

Table 3 Summary of phylogenetic linear regression analyses for the correlations between leaf nutrient concentrations in a tropical karst seasonal rainforest

	Ca	N	P	K	Mg	S	Fe	Cu	Zn
Ca									
N	0.273								
P	0.270	1.103***							
K	1.282***	0.491	0.179						
Mg	1.551***	0.111	0.083	0.866***					
S	0.220	0.946***	0.752***	0.007	0.095				
Fe	0.279	0.948***	0.772***	0.208	0.137	0.714***			
Cu	0.413	0.838*	0.793**	0.083	0.337	0.881***	0.919***		
Zn	0.429	1.098***	0.911***	0.237	0.226	0.872***	0.655**	0.554***	

Slope estimates are given in the lower left section of the matrix (response variable is column 1, explanatory variable in row 1)

Nutrients that are significantly correlated are marked: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

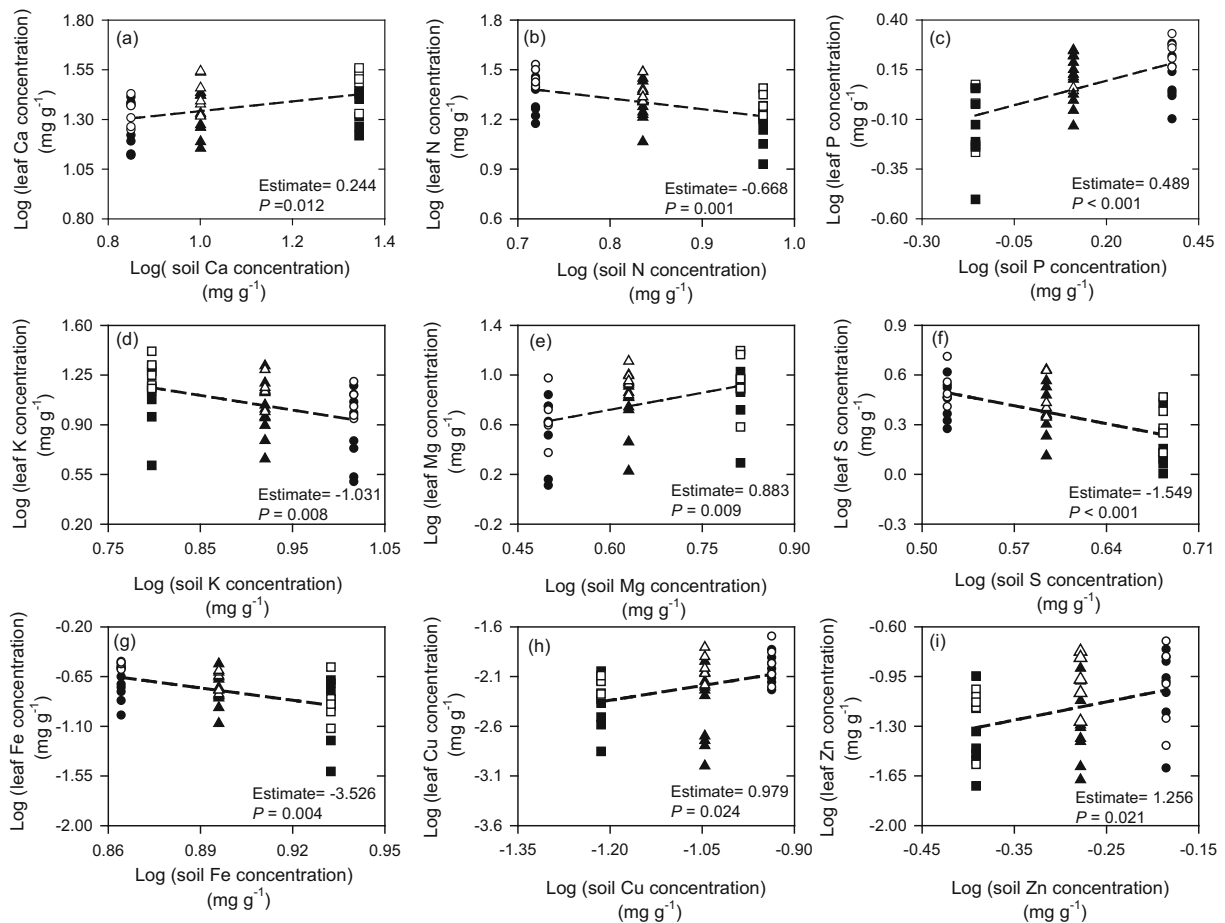


Fig. 5 Correlations between leaf and soil nutrient concentrations for (a) Ca, (b) N, (c) P, (d) K, (e) Mg, (f) S, (g) Fe, (h) Cu and (i) Zn across the evergreen (close symbols) and deciduous species (open symbols) along the continuum of mountain valley (circles).

concentrations were derived from angiosperm species under nutrient-replete conditions and thus could represent the optimal nutrient requirements in leaves of angiosperms. The tight relationship between leaf nutrient concentration in tropical karst tree species and standard leaf nutrient concentration in angiosperms species suggests that variations in leaf nutrient concentrations in tropical karst tree species could be regulated by their differences in optimal requirements. On the other hand, we found the CV of different nutrients in leaves varying from 27.2% for Ca to 58.5% for Zn, indicative of the large intra-nutrient variability (Table 1). According to the stability of limiting nutrient hypothesis (Han et al. 2011), nutrients with higher internal concentrations tend to be more stable and less sensitive to environmental gradients. We found a significant and negative relationship between mean concentration and CV for nutrients

slope (triangles) and peak (squares) in a tropical karst seasonal rainforest. The slope estimates and significant values from phylogenetic linear regression analyses are shown

in leaves (Fig. 2). It's noteworthy that the nutrient CVs in leaves in our study were the result of the combined effects of species and microhabitats. Therefore, nutrients with higher concentrations in tropical karst tree species were more stable and less sensitive to the local valley-slope-peak continuum, consistent with our prediction and supporting the stability of limiting nutrient hypothesis.

Leaf nutrient concentrations exhibited different nutritional statuses in the tropical karst seasonal rainforest. We found mean leaf P concentration below the physiological requirement and only approximated to 20% of the standard concentration (Supporting information Table S2), suggesting that P could be deficient in tropical karst tree species. We found the mean concentrations of N, K, S and Cu in leaves bordering the sufficient concentrations or below the standard concentrations

(Supporting information Table S2), suggesting that N, K, S and Cu could be adequate for growth in tropical karst tree species. We found mean leaf Ca, Mg, Fe and Zn concentrations larger than the standard concentrations but below the toxic concentrations (Supporting information Table S2), suggesting that Ca, Mg, Fe and Zn could be highly accumulated but not toxic in tropical karst tree species. Therefore, the co-existence of leaf Ca hyperaccumulation and P limitation in tropical karst plants is consistent with our prediction and other observations in subtropical karst woody species (Liu et al. 2014; Medina et al. 2017) and tropical and subtropical karst herbs (Hao et al. 2015).

Effect of leaf habit on leaf nutrient concentrations in tropical karst tree species

Since the predominance of evergreen species in low fertility soils was first documented (Monk 1966), numerous studies have discussed the adaptive significance of evergreen species under low-nutrient conditions (e.g., Givnish 2002; Álvarez-Yépiz et al. 2017). Such discussion to date is largely limited to N and P. The concentrations of leaf N and P, together with leaf lifespan, leaf mass per area and photosynthetic and respiration rates, are the components of the universal leaf economics spectrum (Wright et al. 2004). Consistent with previous studies (e.g., Wright et al. 2004; Bai et al. 2015), we found that evergreen species had lower leaf N and P concentrations than deciduous species in tropical karst environments (Table 2). The lower leaf N and P concentrations, together with higher leaf dry mass investment, lower photosynthetic and respiration rates and longer leaf lifespan constitute the conservative leaf strategy in evergreen species (Wright et al. 2004; Donovan et al. 2011). The conservative leaf strategy in evergreen species could help them to be highly competitive and dominate in the P-limited tropical karst environments where natural selection favors traits for their positive role in nutrient conservation (Wright et al. 2004; Pornon et al. 2011).

Beyond N and P, other nutrients are also of importance for plant growth. To extend our knowledge of nutrient fluxes across the co-existing evergreen and deciduous species, attention should be paid to the full set of nutrients (Ågren 2008; Han et al. 2011; Yang et al. 2017). We found the lower concentrations of leaf K, Ca, Mg, S, Fe, Cu and Zn in evergreen species than those in deciduous species (Table 2), as also observed in

subtropical karst plants (Liu et al. 2014) and Chinese plants (Han et al. 2011). Then, what's the role of these nutrients in the success of evergreen species in tropical karst environments? On one hand, we found that the concentrations of leaf S, Fe, Cu and Zn tended to correlate strongly with the concentrations of leaf N and P (Table 3), suggesting that the concentrations of S, Fe, Cu and Zn could be closely connected to the components of leaf economics spectrum (i.e., leaf N and P concentrations). These nutrients are associated with the major constituents of organic material (i.e., N and S), esterification with alcohol groups (i.e., P) and chelation with the prosthetic groups of enzymes (i.e., Fe, Cu and Zn) in leaves (Kirkby 2012; Grusak et al. 2016). We argue that the lower concentrations of these nutrients in evergreen species could decrease the nutrient demands for the metabolic and protective processes and extend their long-term nutrient use efficiency through the formation of long-lived leaves.

On the other hand, we found the concentrations of leaf K, Ca and Mg correlated weakly with the concentrations of leaf N and P (Table 3). K, Ca and Mg are involved in the regulation of the osmotic and electrochemical potentials, enzymatic activation and membrane permeability in leaves (Kirkby 2012; Grusak et al. 2016). We argue that the lower concentrations of leaf K, Ca and Mg in evergreen species reflected the lower nutrient demands for the osmotic, enzymatic and electrochemical processes and that this could lead to the lower mineral investments to maintain cell wall and membrane integrity and stabilize the cell wall chemistry in evergreen species (Sardans and Peñuelas 2015; White et al. 2018).

Effect of soil chemistry on leaf nutrient concentrations in tropical karst tree species

We found that the concentrations of soil Ca, N, Mg, S and Fe tended to increase while the concentrations of soil P, K, Cu and Zn decreased along the valley-slope-peak continuum (Table 4). However, the correlations between leaf and soil nutrient concentrations varied among nutrients along the valley-slope-peak continuum.

Consistent with our prediction and other studies (Han et al. 2011; Hao et al. 2015; Yang et al. 2017), we found strong positive correlations between leaf and soil nutrient concentrations for Ca, Mg, P, Cu and Zn along the valley-slope-peak continuum (Fig. 5). The tight

correlation between leaf and soil nutrient concentrations may be due to the fact that terrestrial plants take up most of their mineral nutrients directly from the soil and the soil nutrient supply is thus a major determinant of nutrient concentrations in plant tissues (Ordoñez et al. 2009; Yang et al. 2017). However, we should be aware of the great discrepancy between the concentrations of mineral nutrients in soil and the nutrient requirements of plants, because soil can contain high concentrations of mineral nutrients not needed for plant growth and even harmful to plants (White 2012). This discrepancy is supported by the finding that Fe concentration larger than the concentrations of P, Mg and S in soil didn't lead to higher leaf Fe concentration (Tables 1 and 4). This discrepancy might be due to the selective ion uptake by which plants could make nutrients required in amounts sufficient to fulfill specific biochemical functions rather than absorb all the available amounts in soil (White 2012).

Along the valley-slope-peak continuum, we also found the negative correlations between leaf and soil nutrient concentrations for N, S, K and Fe (Fig. 5), which could be explained as follows. Soil nutrient concentrations are rough estimates of nutrient supply to the vegetation, because only a proportion of the total nutrient amounts in soil can be taken up and utilized by plants (Marschner and Rengel 2012). The magnitude of this available fraction is strongly affected by soil pH, because soil pH can determine the soil processes including nutrient decomposition, cycling and uptake by plants (Aerts and Chapin 2000; Ordoñez et al. 2009; White 2012). In a typical tropical karst seasonal forest, soil pH tends to increase from acidity to alkalinity along the valley-slope-peak continuum (Chen 1988). We also found a similar trend of soil pH in our study site (data not shown). Low soil pH enhances the availability of Fe in soil due to the changes in oxidation state and solubility (White 2012) and this could result in the higher leaf Fe concentration in the valley (Fig. 5). In contrast, high soil pH facilitates the uptake of K, especially in the Ca-rich soil, because Ca often has an ameliorating effect on the K uptake by plants (White 2012); this could result in the higher leaf K concentration at the peak (Fig. 5). The increase of soil pH can decrease the decomposition of organic matter and this makes certain amounts of N and S immobilized in the organic matter (Aerts and Chapin 2000; Ordoñez et al. 2009). The increase of soil pH can also inhibit the uptake of inorganic N and S forms (i.e., nitrate and sulphate) by plants (White 2012).

Collectively, the increase of soil pH could decrease the availability of organic and inorganic N and S in soil and this resulted in the lower leaf N and S concentrations at the peak (Fig. 5).

Effect of phylogeny on leaf nutrient concentrations in tropical karst tree species

Our results showing the large variations and divergent nutritional statuses in leaf nutrient concentrations and their strong associations with leaf habit and soil chemistry describe the current ecological patterns in the tropical karst environments. The observed ecological patterns were greatly influenced by the evolutionary history of co-existing species, probably because leaf nutrient concentrations changed during species' diversification (Table 1).

Evidence of the phylogenetic effect on leaf nutrient concentrations comes from the detection of a phylogenetic signal (Ackerly 2009). The strong phylogenetic signals in leaf nutrient concentrations of field-collected plants have been reported in various regions (e.g., Watanabe et al. 2007; White et al. 2012; Sardans et al. 2016; Yang et al. 2017). For example, White et al. (2012) found that the relative concentration of many mineral nutrients in plant tissues is determined, to an extent, by the phylogenetic effect at and above species level. The strong phylogenetic effect may result from the species-specific metabolic and physiological use of nutrients, especially of the macro-nutrients, which is due to their long-term adaptation to abiotic and biotic-specific environments (i.e., biogeochemical niche; Sardans and Peñuelas 2014; Yang et al. 2017). However, contrary to our expectation, all the phylogenetic signals in leaf nutrient concentrations were not statistically significant (Table 1). The non-significant phylogenetic signals were also detected for leaf N and P concentrations in tropical dry karst forest species (Fu et al. 2012) and for leaf P, K, Ca, Mg, Fe, Mn and Al concentrations in karst *Primulina* species (Hao et al. 2015). We considered four possible reasons for the differences between studies. First, the phylogenetic signal could depend on the sample species size of the nutrient under investigation. Like any statistic, when sample sizes are large, even small or low values of phylogenetic signal will yield statistically significant *p*-values; thus as the number of species increases, the ability to detect significant levels of phylogenetic signal increase (Kamilar and Cooper 2013). For example, Metali et al. (2012) found non-significant phylogenetic signals in leaf

N, P, K, Ca and Mg concentrations in 58 tropical tree species in Brunei but significant in hundreds of angiosperms in the global dataset even if the values of phylogenetic signal were lower in the latter. Therefore, the non-significant phylogenetic signals in leaf nutrient concentrations in our study could be related to low sample species size, although the current sample size (i.e., 40 species) was good for the power of the detection of λ (i.e., species sample size greater than 30; Kamilar and Cooper 2013). Second, the phylogenetic signal could vary with phylogenetic scale. Measurements of phylogenetic signal in a biological trait may vary at different phylogenetic scales (Kamilar and Cooper 2013). In many cases, the higher phylogenetic scale can lead to the stronger phylogenetic signal (Cavender-Bares et al. 2006). For instance, the significant phylogenetic signals in leaf Ca, K, Mg, Fe, Mn, Al and P concentrations were detected in the Didymocarpoideae subfamily but not in the *Primulina* genus (Hao et al. 2015). However, a greater phylogenetic scale may also decrease phylogenetic signal if between-clade convergence has occurred (Losos 2008). Our one-way analysis of variation on leaf nutrients in the families with at least two species suggests that leaf concentration of all nutrients (but Mg and Fe) was not significantly affected by family (Supporting information Table S3). Furthermore, the similar values of leaf Mg and Fe concentrations were also exhibited in distantly related families. For instance, leaf Mg concentration differed little between Bignoniaceae (6.36 mg g^{-1}) and Meliaceae (6.73 mg g^{-1}) and leaf Fe concentration varied little between Lamiaceae (0.231 mg g^{-1}) and Phyllanthaceae (0.241 mg g^{-1}) (Supporting information Table S3). Therefore, the strong clad convergence at the family level could lead to the non-significant phylogenetic signals in leaf nutrient concentrations in our study. Third, the phylogenetic signal could depend on the variability of leaf nutrient data among related species. For leaf nutrient concentrations, a phylogenetic signal may arise if closely related species exhibit similar trait values (Metali et al. 2012). However, leaf nutrient data are very noisy and variable in nature. We found many cases of high variation ($\text{CV} > 15\%$) in leaf nutrient concentrations for species within the same families that included at least two species (Supporting information Table S3). For example, for species within the Phyllanthaceae family, we found about 2-fold difference between the highest (35.28 mg g^{-1} in *Flueggea virosa*) and lowest (18.19 mg g^{-1} in *Bischofia javanica*) leaf Ca concentration, leading to the high CV (23%) of leaf Ca

concentration. This strong variability of leaf nutrient concentrations within the same family could lead to the closely related species exhibiting notably different nutrient levels and weaken the phylogenetic signals in leaf nutrient concentrations (Blomberg et al. 2003). Finally, the relationship between phylogeny and leaf nutrient concentrations could be mediated by soil nutrients. For example, Verboom et al. (2017) have provided evidence for the significant associations between low-nutrient soils and the predominant distributions of fynbos-specialist lineages that have consistent low leaf nutrient concentrations in the Cape Floristic Region. Our field observations suggest that species distribution along the valley-slope-peak continuum in our study site shows strong species-habitat associations (Guo et al. 2017). For example, *Cephalomappa sinensis* tends to dominate in the slope while *Diospyros siderophylla* tends to dominate at the peak. Therefore, the topography-related species might have been subjected to similar selective pressures related to the soil pH- and Ca- associated factors, leading to the strong edaphic effect on leaf nutrient concentrations (Fig. 5). The strong edaphic effect might imply that distantly-related species could have similar values of leaf nutrient concentrations and converge in their microhabitats in terms of soil conditions and this could reduce the phylogenetic signals in leaf nutrient concentrations. Furthermore, the relative strength of edaphic versus phylogenetic control can vary among individual mineral nutrients (Watanabe et al. 2007; White et al. 2012) and this is likely a consequence of differences in the plant taxon sets and ecosystems studies (Verboom et al. 2017). For example, leaf concentrations of N and P displayed the highest level of phylogenetic signal in our study (Table 1), consistent with leaf P concentration as the strongest indicator of phylogenetic provenance in fynbos-specialist lineages under soil P-limited conditions (Verboom et al. 2017) but in contrast with shoot Ca and Mg concentrations as the strongest indicators of phylogenetic provenance in herbs under fertilizer treatments (White et al. 2012).

Other evidence of the phylogenetic effect on leaf nutrient concentrations comes from the detection of evolutionary processes and rates (Revell et al. 2008; Hernández et al. 2013), which has had little attention in previous studies. The BM model of a random walk provides the foundation for the family of alternative evolutionary models that may incorporate stabilizing selection and time-dependent changes in model parameters (Ackerly 2009). We found that the evolution of leaf

nutrient concentrations could be neither a pure random genetic drift process (i.e., BM model) nor a directional random genetic drift process (i.e., Pagel's directional model), because the two models underperformed other models such as Pagel's δ model and the combination of genetic drift and stabilizing selection (i.e., OU model; Table 1). The weak possibility of Pagel's directional model suggests that the evolution of leaf nutrient concentrations was not likely to begin with an ancestor with the lowest or highest levels of nutrients (i.e., directional change model with positive trend or negative trend). We found that the Pagel's δ model outperformed BM model and the δ values tended to be greater than unity for leaf nutrient concentrations (Table 1), suggesting that leaf nutrient concentrations could show strong species-specific adaptations (Pagel 1999; Hernández et al. 2013). However, we found the general lowest AICs values in OU model (Table 1), suggesting that the OU model could best describe the evolution of leaf nutrient concentrations in tropical karst environments. Under the OU model, the evolution of leaf nutrient concentrations likely began with an ancestor which had adaptive nutrient levels to allow it to survive in tropical karst environments. For example, an ancestor with high leaf Ca accumulation was adaptive in karst soils; but, if leaf Ca accumulation was extremely high or low, it would lead to the malfunction in cell wall chemistry and cytosolic signalling in leaves (White and Brown 2010; White et al. 2018). During species' diversification, stabilizing selection towards an optimum value could constrain the variation in leaf nutrient concentrations, which could both eliminate species with extremely high or low leaf nutrient concentrations and make distantly related species to show similar values of leaf nutrient concentrations. Therefore, species specification and stabilizing selection could lead to specific adaptations to the tropical karst environments. All these adaptive trajectories were likely to produce an overlapping ranges of leaf nutrient concentrations among species, especially for species within the same functional group (e.g., evergreen species) or species growing in the same microhabitat (e.g., species coexisting at the peak) (Fig. 5). Consistent with our prediction, we found that the values of sig^2 , α , δ and absolute drift varied greatly among leaf nutrients (Table 1), suggesting the obvious variability of evolutionary rate among leaf nutrients. The great variability of evolutionary rate among leaf nutrients under the BM model was also observed in plant lineages growing in nutrient-limited soils (Verboom et al. 2017).

The faster rates of some leaf nutrients under a given evolutionary model could help closely related species rapidly diversify to fill new environments where the quick adjustments to adaptive nutrient levels could be preferential for plant fitness (Bai et al. 2015). In addition, evolutionary process, rate and phylogenetic signal are not mutually independent. Actually, the strength of phylogenetic signals in leaf nutrient concentrations should be the result, at least in part, of the long-term evolution. The non-significant phylogenetic signals in leaf nutrient concentrations reflected non-BM evolutionary processes (Table 1). Moreover, consistent with our prediction and previous investigations on plant functional traits (Ackerly 2009; Bai et al. 2015), we found the close relationship between evolutionary rate and signal across leaf nutrient concentrations (Fig. 3). However, such relationship varied depending on the model of evolution, because we found the weakly negative relationship between λ and absolute drift but strongly negative relationship between λ and sig^2 , α or δ (Fig. 3). Specially, the strongly negative relationship between λ and α could be related to the fact that stabilizing selection towards an optimum value pulls all species back toward the central point and erase the signal of their prior history (Ackerly 2009).

Although leaf nutrient concentrations had different levels of phylogenetic signal and evolutionary rates, we should be aware that the phylogenetic effect could lead to the strong coupling of leaf nutrients during species' diversification. Leaf N, P, S, Fe, Cu and Zn concentrations strongly and positively correlated with each other, as did for leaf Ca, Mg and K concentrations (Table 3 and Fig. 4). The strong positive correlations between leaf concentrations of mineral nutrients have been also observed in tropical and subtropical karst *Didymocarpoideae* species (e.g., Ca and K vs Mg; Hao et al. 2015) and subtropical karst woody species (e.g., N vs P, Ca vs Mg; Medina et al. 2017). The strong cross-species correlations suggest that there is a phylogenetic effect limiting the independent evolution of leaf nutrient concentrations (Wright et al. 2004; Donovan et al. 2011). In other words, the phylogenetic effect could limit the evolution of the linked nutrients if the co-existing tree species lack the phylogenetic variation necessary to produce the fit nutrient combinations (Broadley et al. 2004; Donovan et al. 2011). However, we also found some weak cross-species correlations between leaf nutrient concentrations (Table 3). For instance, leaf Ca concentration correlated weakly with concentrations of leaf N P, S, Fe, Cu and Zn. The weak

relationships of leaf Ca concentration with leaf N, P and Fe concentrations were also observed in subtropical karst woody species (Medina et al. 2017). The observed coupling and decoupling of leaf nutrient concentrations support our prediction that the strength of interactions differ among leaf nutrients, which could be constrained by the divergent biochemical functions among leaf nutrients (Kirkby 2012; Grusak et al. 2016). Nutrients with identical biochemical function have similar ion uptake mechanism and thus tend to be closely inter-correlated (Kirkby 2012). For example, leaf Ca and Mg concentrations among angiosperm species are positively correlated due to their essential role in determining cell wall cation exchange capacity (Table 3 and Fig. 4; White et al. 2018; Neugebauer et al. 2018). However, it's noteworthy that the strength of interactions among leaf nutrient concentrations can vary with the environmental changes. For example, the correlation between leaf K and P concentrations was weak in our study (Table 3) and in subtropical karst woody species (Medina et al. 2017) but very strong in plant lineages growing in the Cape Floristic Region (Verboom et al. 2017). According to Donovan et al. (2011), the evolution of leaf trait combinations can be considered as selection by eliminating species with combinations of leaf traits that function poorly in a given environment. The coupling of leaf nutrients could be considered as the selected target in order to eliminate species with poor nutrient combinations in leaves in a given environment. For example, species with high Ca concentration but low Mg concentration in leaves might be selected against because cell wall chemistry and the Mg/Ca mass quotients in cell walls might be malfunctioning in these leaves (White et al. 2018; Neugebauer et al. 2018). Therefore, selection combined with the biochemical constraints would maintain certain leaf nutrient combinations that should be well-fitted for tree species growing in tropical karst environments.

Conclusions

Our results demonstrate that tree species had large inter- and intra-nutrient variability and were characterized by the combination of P limitation and Ca hyperaccumulation in leaves in tropical karst environments. Compared with deciduous species,

evergreen species had lower nutrient concentration requirements to fulfill specific biochemical functions in leaves. Specifically, evergreen species had lower concentrations of mineral nutrients associated with the major constituents in plant organic material (i.e., N and S), esterification with alcohol groups (i.e., P), chelation with the prosthetic groups of enzymes (i.e., Fe, Cu and Zn) and the regulation of the osmotic and electrochemical potentials, enzymatic activation and membrane permeability (i.e., K, Ca, Mg) in leaves. Along the valley-slope-peak continuum, we found that the correlations between leaf and soil nutrient concentrations were positive for Ca, Mg, P, Cu and Zn and negative for N, S, K and Fe. We found the general non-significant phylogenetic signals in leaf nutrient concentrations, which could result from their large nutrient variability and strong associations with soil conditions. Non-significant phylogenetic signals were also closely related to evolutionary processes. We found that the Pagel's δ model general outperformed BM model and the δ values were larger than unity for leaf nutrient concentrations, suggesting the importance of species-specific leaf nutrient adaptations during species' diversification. However, the evolution of leaf nutrient concentrations was best fitted by the OU model which could pull all species back toward the central point and erase the signal of their prior history. Moreover, we found that phylogenetic signal tended to be negatively associated with evolutionary rate across leaf nutrient concentrations, although evolutionary rate under a given evolutionary model varied greatly among leaf nutrient concentrations. We found both the coupling and decoupling of leaf nutrients among leaf nutrient concentrations, which were strongly constrained by the divergent biochemical functions among leaf nutrients. Taken together, stabilizing selection combined with the biochemical constraints could select the locally adapted evergreen and deciduous species with sufficient phylogenetic variations to produce leaf nutrient concentrations and certain nutrient combinations that should be well-fitted in tropical karst environments.

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