

Concentrations and resorption patterns of 13 nutrients in different plant functional types in the karst region of south-western China

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- **Background and Aims** Elucidating the stoichiometry and resorption patterns of multiple nutrients is an essential requirement for a holistic understanding of plant nutrition and biogeochemical cycling. However, most studies have focused on nitrogen (N) and phosphorus (P), and largely ignored other nutrients. The current study aimed to determine relationships between resorption patterns and leaf nutrient status for 13 nutrient elements in a karst vegetation region.
- **Methods** Plant and soil samples were collected from four vegetation types in the karst region of south-western China and divided into eight plant functional types. Samples of newly expanded and recently senesced leaves were analysed to determine concentrations of boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), N, sodium (Na), P, sulphur (S) and zinc (Zn).
- **Key Results** Nutrient concentrations of the karst plants were lower than those normally found in other regions of China and the rest of the world, and plant growth was mainly limited by P. Overall, four nutrients revealed resorption [N (resorption efficiency 34.6%), P (48.4%), K (63.2%) and Mg (13.2%)], seven nutrients [B (–16.1%), Ca (–44.0%), Cu (–14.5%), Fe (–205.5%), Mn (–72.5%), Mo (–35.6%) and Zn (–184.3%)] showed accumulation in senesced leaves and two nutrients (Na and S) showed no resorption or accumulation. Resorption efficiencies of K and Mg and accumulation of B, Ca, Fe and Mn differed among plant functional types, and this strongly affected litter quality. Resorption efficiencies of N, P and K and accumulation of Ca and Zn increased with decreasing concentrations of these nutrients in green leaves. The N:P, N:K and N:Mg ratios in green leaves predicted resorption proficiency for N, K and Mg, respectively.
- **Conclusions** The results emphasize the fact that nutrient resorption patterns strongly depend on element and plant functional type, which provides new insights into plant nutrient use strategies and nutrient cycling in karst ecosystems.

Key words: karst ecosystem, multiple nutrients, nutrient concentration, nutrient limitation, plant functional type, plant nutrient use strategy, resorption efficiency, resorption proficiency, stoichiometry.

INTRODUCTION

Plants require multiple nutrients and generally acquire them from the soil solution. Some nutrients are required in large quantities and others in only small amounts (Marschner, 1995; Ågren, 2008; White and Brown, 2010). According to ecological stoichiometry, it is essential to maintain sufficient contents and stable proportions of multiple nutrients in plant tissues for healthy growth (Elser *et al.*, 2000b; Sterner and Elser, 2002). Nitrogen (N) and phosphorus (P) are critical components of plant nutrition and globally are considered to be the most important nutrients limiting plant growth and carbon storages in terrestrial ecosystems (Elser *et al.*, 2007). Accordingly, most studies on nutrient stoichiometry and resorption patterns have focused on N and P, while other nutrients have been poorly investigated (Lynch and St Clair, 2004; Watanabe *et al.*, 2007; Ågren, 2008; Han *et al.*, 2011). However, a biogeochemical process may be co-limited by multiple nutrients and/or may experience shifting degrees of limitation by different elements at multiple timescales (Vitousek, 2004; Townsend *et al.*, 2011).

Many nutrients other than N and P also play important roles in plant metabolic processes and ecosystem functioning. Many inorganic ions take part in photosynthesis, osmotic adjustment and antioxidant protection (Marschner, 1995). Mineral nutrients can influence plant growth through both limitation and toxicity. Toxicities of manganese (Mn) and aluminium commonly occur on acid soils (White and Brown, 2010), while alkaline soils are characterized by poor availabilities of P, iron (Fe), copper (Cu), Mn and zinc (Zn) (Lynch and St Clair, 2004). Recent studies suggested that multiple nutrients may co-regulate the carbon cycle, by affecting the functions and processes of ecosystems (Townsend *et al.*, 2011). For example, many micronutrients shape litter production and decomposition rate (Kaspari *et al.*, 2008). Manganese enhances lignin degradation in the litter of many trees, such as oaks (Davey *et al.*, 2007) and pines (Berg *et al.*, 2010), while calcium (Ca) can affect tree growth and litter production (Paoli and Curran, 2007) and forest floor turnover rate (Reich *et al.*, 2005). Molybdenum (Mo) can promote N fixation in tropical forests (Barron *et al.*, 2009). These results highlight that studies of stoichiometric relations and the

relative needs and limitations of multiple nutrients are urgently needed for a holistic understanding of the ecology of plant nutrition (Ågren, 2008; Han *et al.*, 2011).

Nutrient resorption is one of the most important strategies employed by plants to conserve nutrients, especially in low-nutrient environments (Aerts, 1996; Killingbeck, 1996), and affects many ecosystem processes, such as nutrient uptake, litter decomposition, plant competition and carbon cycling (Killingbeck, 1996; Yuan and Chen, 2009a; Vergutz *et al.*, 2012). Thus, nutrient resorption parameters are essential for the accuracy of ecosystem and biogeochemical models (Thornton *et al.*, 2007; Reed *et al.*, 2012; Vergutz *et al.*, 2012). Nutrient resorption can be quantified as resorption efficiency (RE) and resorption proficiency (RP) (Aerts, 1996; Killingbeck, 1996). Resorption efficiency (defined as percentage reduction of nutrient concentration between green and senesced leaves) is best suited to quantifying the relative degree to which plant can conserve nutrients invested in foliage. Resorption proficiency (defined as the terminal nutrient concentration in senesced leaves) appears to be a more definitive and objective measure of the degree to which selection has acted to minimize nutrient loss (Killingbeck, 1996).

Nutrient resorption usually varies among plant functional types. For example, the RE of N is higher in deciduous species and graminoids than in evergreen species and forbs (Aerts, 1996), and higher in shrubs than in trees (Yuan and Chen, 2009a). Graminoids have higher REs of P, K, Ca and Mg than evergreen and deciduous angiosperms (Vergutz *et al.*, 2012). These variations can be attributed to differences in habitat, leaf lifespan and the size of the non-leaf nutrient pool (Kull and Kruijt, 1999; Wright and Westoby, 2003; Kobe *et al.*, 2005; Vergutz *et al.*, 2012). As nutrient resorption is considered to be particularly important in low-fertility soils, previous studies have extensively investigated the relationships between nutrient resorption and nutrient availabilities. Some studies found no clear relationships (Aerts, 1996; Wright and Westoby, 2003; Kazakou *et al.*, 2007), while others suggested that nutrient RE depended on plant or soil nutrient status (Kobe *et al.*, 2005; Rejmánková, 2005; Ratnam *et al.*, 2008; Vergutz *et al.*, 2012). When nutrient availability was expressed as relative nutrient limitation (e.g. N:P ratio in green leaves), nutrient RP seemed to be more sensitive than RE (Rejmánková, 2005; Ratnam *et al.*, 2008). To date, whether and how nutrient resorption is regulated by plant nutrient status across plant functional types and ecosystems remain unresolved (Aerts *et al.*, 2007; Ratnam *et al.*, 2008; Vergutz *et al.*, 2012). Moreover, most research has only addressed patterns of N and P resorption, while resorption patterns of other essential nutrients have seldom been reported – but see Hagen-Thorn *et al.* (2006) and Vergutz *et al.* (2012). It was reported that macro- and micro-nutrient concentrations in plants can be changed by experimental drought and warming, and that the changes depended on the nutrient and species (Sardans *et al.*, 2008), which could affect plant nutrient use strategies, plant growth and ecosystem processes. This emphasizes the importance of elucidating the resorption patterns of multiple nutrients in accurately modelling biogeochemical cycling and ecosystem productivity, especially under global change (Aerts *et al.*, 2007; Reed *et al.*, 2012; Vergutz *et al.*, 2012).

Karst topography is widespread throughout the world and covers about 12 % of the world's land area (Liu, 2009). The karst landscape of south-western China is one of the most typical landscapes developed on carbonate bedrock in the world,

and is characterized by extremely slow soil formation from the underlying limestone and very shallow and patchy soil with a low water retention capacity (Zhu, 1997; Liu, 2009). The typical vegetation in the subtropical karst region is a mixed evergreen and deciduous broad-leaved forest. Due to human disturbances, many karst forests have experienced varying degrees of degradation. Many vegetation types, such as forest, shrub forest, shrubland and grassland, occur in this region, and the grasslands are the result of serious degradation of other vegetation types. For a long time, water availability was considered to be the most important factor limiting plant growth and vegetation restoration in this karst region (Zhu, 1997; Liu, 2009). However, recent studies have suggested that shortage of mineral nutrients resulting from the limited total soil mass could be a crucial factor limiting plant growth and ecosystem productivity in this region (Zhang and Wang, 2009; Du *et al.*, 2011; Guo *et al.*, 2011). It was reported that the mean depth of topsoil on the karst hills was only about 2–9 cm (Zhang and Wang, 2009; Liu *et al.*, 2013). However, nutrient limitations and nutrient conservation strategies of the native plants in this region are still being questioned, and clarification is urgently needed to improve our understanding of plant nutrient use strategies, species compositions, competitive relationships and dynamics of the native vegetation.

The present study investigated the stoichiometry and resorption patterns of 13 mineral nutrients [boron (B), Ca, Cu, Fe, potassium (K), magnesium (Mg), Mn, Mo, N, sodium (Na), P, sulfur (S) and Zn] in different plant functional types from four different vegetation types (forests, shrub forests, shrublands and grasslands) in the karst region of south-western China. We addressed the following questions. (1) What are the relative limiting nutrients in this karst region? We expected that the multiple nutrients that are required in large quantities would co-limit plant growth in the karst region, due to the shallow soil layer. Phosphorus would probably be the most important limiting factor because of the lowered mobility of P in calcareous soils. (2) How does plant nutrient status influence nutrient resorption across plant functional types? It was expected that nutrient resorption values would increase as nutrient concentrations in green leaves decreased or nutrient relative limitations increased. This response would also vary among functional types. (3) How do resorption patterns differ among mineral nutrients? We hypothesized that nutrients required in large quantities would be resorbed more effectively than nutrients required in small amounts. Magnesium and Ca would be less resorbed than other nutrients, such as N, P and K, because calcareous soils are usually rich in Mg and Ca. (4) How does vegetation degradation affect nutrient stoichiometry and resorption patterns? We expected that soils would become more sterile during the process of deforestation and therefore plant nutrient concentrations would be lower and nutrient resorption would be more effective in grasslands than in forests.

MATERIALS AND METHODS

Study region

The research was carried out at the Puding Karst Ecosystem Research Station of the Chinese Academy of Sciences in Guizhou Province, China (26°09'–26°31'N, 105°27'–105°58'E). The mean annual precipitation and temperature of this region are 1390 mm and 15.1 °C, respectively. The topsoil

in this region is extremely shallow and patchy, in the range of 0–30 cm deep. The typical vegetation is mixed evergreen and deciduous broad-leaved forest. Due to human disturbance, four main vegetation types occur in this region: mixed evergreen and deciduous broad-leaved forests, shrub forests, shrublands and grasslands.

Platycarya longipes, *Carpinus pubescens*, *Lithocarpus confinis* and *Itea yunnanensis* dominate the forest vegetation. The mean height of canopy trees is about 15 m and the mean canopy coverage is about 90 %. The shrub forest vegetation is dominated by *P. longipes* and *I. yunnanensis*, accompanied by many thorny shrubs such as *Rosa cymosa* and *Rhamnus heterophylla*. The mean height of the canopy trees in shrub forest vegetation is about 6 m. The shrubland vegetation is dominated by many thorny shrub species (2 m height), such as *Ro. cymosa*, *Rh. heterophylla*, *Zanthoxylum planispinum* and *Pyracantha fortuneana*. The grassland vegetation is dominated by *Themeda japonica* and *Heteropogon contortus* and contains some dwarf shrubs. More details about the four vegetation types were described by Liu et al. (2011). Nutrient contents of topsoil of the four vegetation types are described in Supplementary Data Table S1. The main differences among the vegetation types are the consequences of human-induced vegetation degradation. The forest vegetation is considered a reference for the natural vegetation in this region and shows little disturbance, while shrubland and grassland vegetation are the results of intensive disturbances.

Sampling and chemical analysis

Plant and soil samples were collected from the four vegetation types, with each type having three studied sites (Supplementary Data Table S2). According to previous vegetation inventories of sampling plots in these sites (Liu et al., 2011), we collected mature green and senesced leaves of dominant and common species in each sampling plot. New fully expanded and intact green leaves were sampled during July and August 2011, and recently senesced tree and shrub leaves and standing dead herb leaves were sampled during November and December. Mature green leaves situated at the same height were taken at 4–5 m for trees, 1–2 m for shrubs and 0.1–0.3 m for herbs. For each species at each site, three to five samples were randomly collected from different healthy individuals. Because many species co-occur in different sites, we labelled each species at each site as a ‘site species’. In total we collected 172 site species, which belonged to 64 plant species, with matched pairs of green and senesced leaves. Sampled species were classified into eight plant functional types: six ferns, 18 forbs, 12 grasses (Cyperaceae and Gramineae), 34 evergreen shrubs, 22 evergreen trees, 28 deciduous shrubs, 37 deciduous trees and 15 lianas (Supplementary Data Table S3). Five topsoil samples (0–20 cm) per site were also collected simultaneously with sampling the green leaves. All samples were air-dried in the field before being shipped to the laboratory for analysis. Total N concentration was analysed using the Kjeldahl method and concentrations of other nutrients (B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S and Zn) were determined with an inductive coupled plasma emission spectrometer after digestion of the samples in concentrated HNO₃.

Data analysis

To determine the relative limitations of multiple nutrients, we used the physiological concentration requirements (Marschner,

1995; Han et al., 2011) and the optimal ratios of multiple nutrients (Linder, 1995; Knecht and Göransson, 2004). According to Marschner (1995) and Han et al. (2011), the physiological concentration requirements of N, K, Ca, Mg, P, S, Fe, Mn, Zn, B, Cu and Mo for adequate plant growth are 15, 10, 5, 2, 2, 1, 0.1, 0.05, 0.02, 0.02, 0.006 and 0.0001 g kg⁻¹, respectively. According to Linder (1995), the optimal ratios of multiple nutrients are: N:P:K:Ca:S:Mg:Fe:Mn:Zn:Cu:B:Mo = 100:10:35:2.5:5:4:0.2:0.05:0.05:0.03:0.05:0.007. Concentrations of the other 11 nutrients in green leaves were plotted against N concentrations and a line representing the optimal ratio was added. As N is required in large amounts and commonly limits plant growth in terrestrial ecosystems, we judged relative N limitation or limitations of other nutrients in the karst plants in relation to the optimal ratios.

Mean nutrient concentrations in green ([nutrient]_{green}) and senesced ([nutrient]_{sen}) leaves for each species at each site were calculated from individual samples. Nutrient resorption was expressed as RE and RP. Because we could not match [nutrient]_{green} and [nutrient]_{sen} at the individual level, we calculated nutrient RE for different plant functional types based on the mean nutrient concentrations at species level at each site. We used the method of Kobe et al. (2005) to calculate RE for different functional types and the whole data set, and to determine the effect of plant nutrient status on RE:

$$[\text{nutrient}]_{\text{sen}} = \alpha([\text{nutrient}]_{\text{green}})^{\beta} \quad (1)$$

The parameter α is an index of nutrient RE, while exponent β determines how [nutrient]_{green} controls RE. When $\beta = 1$, the nutrient status of green leaves has no effect on RE. In this case, RE is equal to $(1 - \alpha) \%$. When $\beta > 1$, RE decreases with increasing nutrient concentration, while $\beta < 1$ implies increasing RE with increasing nutrient concentration (Kobe et al., 2005). When $\beta \neq 1$,

$$\text{RE} = 1 - \frac{\alpha([\text{nutrient}]_{\text{green}})^{\beta}}{[\text{nutrient}]_{\text{green}}} \times 100\% \quad (2)$$

Log₁₀ transformation of equation 1 yields the linear form:

$$\log_{10}([\text{nutrient}]_{\text{sen}}) = \alpha' + \beta \times \log_{10}([\text{nutrient}]_{\text{green}}) \quad (3)$$

where α' is $\log_{10} \alpha$ and α' and β are estimated from the data. To determine the effect of [nutrient]_{green} on RE, we fitted eqn (3) using major axis regression, which is appropriate in stoichiometric studies (Kobe et al., 2005; Niklas, 2006). Regression parameters were estimated using the SMATR library (version 2.0; Warton et al., 2006).

The nutrient variables ([nutrient]_{green}, [nutrient]_{sen} and nutrient RE) of a plant community were weighted averages calculated from the leaf mean values of the dominant species and the importance values (IV) of the dominant species in the community:

$$[\text{nutrient variables}]_{\text{community}} = \sum_i^n ([\text{nutrient variables}]_i \times \text{IV}_i) / \sum_i^n \text{IV}_i \quad (4)$$

where [nutrient variables]_{community} are the [nutrient]_{green}, [nutrient]_{sen} and nutrient RE of a plant community, [nutrient

variables]; are the $[\text{nutrient}]_{\text{green}}$, $[\text{nutrient}]_{\text{sen}}$ and nutrient RE of the i th plant species, and IV_i is the importance value of the i th plant species in a plant community.

One-way ANOVA was performed to determine differences among plant functional types or vegetation types. Because of the limited amount of data, data on evergreen shrubs, deciduous shrubs and deciduous trees were subjected to two-way ANOVA to determine the effects of functional type, vegetation type and their interaction on nutrient variables. The significances of differences between means were determined by Tukey's test at $P < 0.05$. The mean nutrient RE of the whole data set and plant functional types was assessed for difference from 0% RE by the t -test. To determine the effects of relative nutrient limitations on nutrient resorption, the correlations between the ratios of green leaf N to other nutrients and nutrient RE and RP were explored by Pearson's correlation analysis and linear regression. To test the effects of soil nutrient contents on leaf nutrient concentrations, the correlations between mean soil nutrient contents of each vegetation type and mean leaf nutrient concentrations and nutrient RE for evergreen shrubs, deciduous shrubs and deciduous trees were also explored by Pearson's correlation analysis. The ratios of N to other nutrients, $[\text{nutrient}]_{\text{green}}$ and $[\text{nutrient}]_{\text{sen}}$ of each species at each site were \log_{10} -transformed to meet assumptions of normality. Statistical analyses were performed with SPSS version 15.0 (SPSS, USA).

RESULTS

Concentrations of multiple nutrients in different plant functional types

The mean concentrations of the 13 nutrients in green leaves of the karst plants varied greatly, ranging from 0.0023 g kg^{-1} for Mo to 19.39 g kg^{-1} for Ca (Table 1), with Ca:N:K:Mg:S:P:Na:Fe:Mn:B:Zn:Cu:Mo = 111:100:59:18:14:8.6:0.85:0.82:0.48:0.18:0.13:0.075:0.013. The relative variability of the 13 nutrient concentrations in green leaves [indicated by their coefficient of variation (CV, %)] increased in the order P (21.6%) < N (25.7%) < Mo (30.0%) < Cu (30.8%) < Fe (38.3%) < B (44.9%) < Mg (51.4%) < Ca (53.6%) < S (57.1%) < K (61.8%) < Na (64.4%) < Zn (68.2%) < Mn (110%). The mean $[\text{nutrient}]_{\text{sen}}$ followed similar orders of magnitude to $[\text{nutrient}]_{\text{green}}$, varying from 0.0028 g kg^{-1} for Mo to 26.09 g kg^{-1} for Ca (Table 1), with Ca:N:K:Mg:S:P:Na:Fe:Mn:B:Zn:Cu:Mo = 231:100:33:24:21:6.8:1.1:3.8:1.2:0.31:0.50:0.12:0.025. The N concentration in senesced leaves showed the smallest CV (29.2%) and Mn the largest (158.1%).

Concentrations of multiple nutrients showed significant differences among plant functional types (Table 1). Deciduous trees had the highest N concentrations in green and senesced leaves, and evergreen trees and shrubs the lowest values. Ferns had the highest P concentrations in green and senesced leaves, while evergreen shrubs had the lowest values. Most of the lowest concentrations of B, Ca, Fe, K, Mg, Mn, S and Z in green and senesced leaves were in grasses or evergreen species, and the highest in forbs and/or lianas. There were no significant differences among functional types in Cu, Mo and Na concentrations in green and senesced leaves.

Relative limitations of nutrients in karst plants

Among the 12 nutrients (B, Ca, Cu, Fe, K, Mg, Mn, Mo, N, P, S and Zn), only mean P concentrations in green leaves of karst plants

(1.5 g kg^{-1} ; Table 1) were significantly lower than the physiological concentration requirement of P (2.0 g kg^{-1} ; t -test, $P < 0.05$). When data were grouped by plant functional type, the P concentration of each functional type was also lower than the physiological concentration requirement (Table 1; $P < 0.05$). Mean K concentrations in green leaves of evergreen and deciduous shrubs and trees ($7.84\text{--}8.47 \text{ g kg}^{-1}$; Table 1) were lower than the physiological concentration requirement of K (10 g kg^{-1} ; $P < 0.05$), while concentrations of the other ten nutrients were generally not lower than the physiological concentration requirements (Table 1).

For P, values mostly fell below the optimal line (Fig. 1). For the other ten nutrients, most values were above the optimal line, with only a few values below the line but still very close to it.

Nutrient RE and RP

Considering all data as a whole, four nutrients (N, P, K and Mg) revealed resorption, while the other nine nutrients tended to accumulate in senesced leaves (Fig. 2). Average nutrient RE increased in the following order: Mg (13.2%) < N (34.6%) < P (48.4%) < K (63.2%). Mean accumulation was greatest for Fe (RE -205.5%) and lowest for S (RE -1.4%). The accumulations of Na and S were not significant (Fig. 2).

Nutrient RE or accumulation differed significantly among functional types (Fig. 2). For N, P and K, resorption was observed in all functional types. RE for N was highest in lianas (41.7%) and lowest in grasses (26.2%). RE for P was highest in deciduous trees (54.7%) and lowest in deciduous shrubs (40.4%). RE for K was higher in ferns, forbs and grasses (77.6–88.3%) than in other functional types. There was significant Mg resorption in grasses, evergreen trees, deciduous shrubs and trees, and lianas, with the highest RE in grasses (39.1%). Only deciduous trees revealed significant S resorption (12.5%), and forbs and evergreen shrubs showed Na resorption (31.1 and 24.6%, respectively).

All functional types showed significant accumulations of B (except for forbs and grasses), Ca (except for grasses), Fe, Mn and Zn (Fig. 2). Accumulations of B (41.8%; RE -41.8%), Ca (123.1%) and Mn (255.0%) were highest in ferns. Grasses showed the highest Fe accumulation (487.6%) and deciduous shrubs the highest Zn accumulation (249.4%). However, Cu accumulation only occurred in forbs and deciduous trees (25.7 and 19.5%, respectively) and Mo accumulation was observed in shrubs and trees (30.8–38.0%).

Because only N, P, K and Mg revealed resorption across all data, we mainly focused on the RPs (concentrations in senesced leaves) of these four nutrients. Across all data, mean RPs of N, P, K and Mg were 1.13, 0.077, 0.38 and 0.27%, respectively (Table 1). The highest RPs for N and P were in evergreen shrubs and the highest RPs for K and Mg were in grasses (Table 1), whereas the lowest RPs for N, P and K were in deciduous trees, ferns and lianas, respectively.

Effect of plant nutrient status on nutrient resorption

Across all data, the values of $[\text{nutrient}]_{\text{sen}}$ were positively correlated with $[\text{nutrient}]_{\text{green}}$ for the 13 nutrients except for Cu, Mo and Na (Supplementary Data Fig. S1). For N, P and K the β value was > 1 ($P < 0.05$; Table 2; Supplementary Data Fig. S1), indicating that REs of N, P and K increased with decreasing

TABLE 1. Nutrient concentrations in green and senesced leaves of different plant functional types (g kg^{-1})

Nutrient	Leaf type	All data ($n = 172$)	Ferns ($n = 6$)	Forbs ($n = 18$)	Grasses ($n = 12$)	Evergreen shrubs ($n = 34$)	Evergreen trees ($n = 22$)	Deciduous shrubs ($n = 28$)	Deciduous trees ($n = 37$)	Lianas ($n = 15$)
Ca	Green	19.39 ± 0.80	8.73 ± 2.49 ^a	12.96 ± 0.72 ^b	6.35 ± 0.66 ^a	24.33 ± 1.66 ^c	10.55 ± 1.10 ^{ab}	24.68 ± 2.03 ^c	23.29 ± 1.51 ^c	22.72 ± 2.12 ^c
	Senesced	26.09 ± 0.94	15.05 ± 2.34 ^b	22.64 ± 2.50 ^{bc}	7.76 ± 0.69 ^a	31.77 ± 1.49 ^c	15.89 ± 1.26 ^b	29.83 ± 1.88 ^c	30.90 ± 2.15 ^c	30.85 ± 2.56 ^c
N	Green	17.51 ± 0.34	15.40 ± 1.39 ^a	17.70 ± 1.13 ^{ab}	15.39 ± 1.33 ^a	15.03 ± 0.51 ^a	15.00 ± 0.51 ^a	17.85 ± 0.79 ^{ab}	20.65 ± 0.71 ^b	20.57 ± 1.22 ^b
	Senesced	11.31 ± 0.25	10.70 ± 2.32 ^{ab}	11.65 ± 1.04 ^{ab}	11.52 ± 1.42 ^{ab}	9.40 ± 0.40 ^a	10.48 ± 0.54 ^{ab}	12.13 ± 0.49 ^b	12.54 ± 0.46 ^b	11.93 ± 0.82 ^{ab}
K	Green	10.34 ± 0.49	16.95 ± 2.01 ^{bc}	20.05 ± 3.00 ^c	12.65 ± 1.89 ^b	7.84 ± 0.52 ^a	8.13 ± 0.99 ^a	8.41 ± 0.69 ^{ab}	8.47 ± 0.54 ^{ab}	12.80 ± 1.03 ^b
	Senesced	3.76 ± 0.23	3.62 ± 1.37 ^{bc}	4.56 ± 1.51 ^c	1.37 ± 0.24 ^a	4.30 ± 0.45 ^c	4.00 ± 0.65 ^{bc}	3.70 ± 0.54 ^{bc}	3.02 ± 0.27 ^b	5.03 ± 0.47 ^c
Mg	Green	3.23 ± 0.13	3.55 ± 0.59 ^{bc}	3.42 ± 0.18 ^{bc}	2.26 ± 0.25 ^a	2.51 ± 0.17 ^{ab}	2.47 ± 0.26 ^a	3.76 ± 0.29 ^{bc}	3.38 ± 0.17 ^{ab}	5.03 ± 0.92 ^c
	Senesced	2.72 ± 0.10	3.61 ± 0.74 ^{cd}	3.30 ± 0.23 ^{cd}	1.32 ± 0.18 ^a	2.37 ± 0.19 ^{bc}	2.15 ± 0.20 ^b	2.89 ± 0.21 ^{bcd}	2.69 ± 0.16 ^{bc}	4.07 ± 0.58 ^d
S	Green	2.48 ± 0.11	2.07 ± 0.28 ^a	2.59 ± 0.31 ^a	2.36 ± 0.45 ^a	2.01 ± 0.09 ^a	2.02 ± 0.23 ^a	2.13 ± 0.20 ^a	2.87 ± 0.32 ^a	3.98 ± 0.44 ^b
	Senesced	2.42 ± 0.09	2.71 ± 0.33 ^a	2.16 ± 0.15 ^a	2.52 ± 0.20 ^a	2.10 ± 0.15 ^a	1.97 ± 0.27 ^a	2.35 ± 0.24 ^a	2.34 ± 0.17 ^a	4.48 ± 0.47 ^b
P	Green	1.50 ± 0.03	1.73 ± 0.10 ^b	1.69 ± 0.10 ^b	1.44 ± 0.08 ^{ab}	1.26 ± 0.04 ^a	1.45 ± 0.06 ^{ab}	1.48 ± 0.04 ^{ab}	1.63 ± 0.06 ^b	1.65 ± 0.07 ^b
	Senesced	0.77 ± 0.02	0.89 ± 0.10 ^b	0.85 ± 0.08 ^{ab}	0.78 ± 0.07 ^{ab}	0.66 ± 0.03 ^a	0.75 ± 0.06 ^{ab}	0.87 ± 0.04 ^b	0.75 ± 0.04 ^{ab}	0.80 ± 0.06 ^{ab}
N ^a	Green	0.15 ± 0.01	0.10 ± 0.02 ^a	0.18 ± 0.02 ^a	0.16 ± 0.04 ^a	0.15 ± 0.02 ^a	0.13 ± 0.01 ^a	0.13 ± 0.02 ^a	0.16 ± 0.02 ^a	0.16 ± 0.02 ^a
	Senesced	0.13 ± 0.01	0.17 ± 0.05 ^a	0.12 ± 0.02 ^a	0.13 ± 0.02 ^a	0.10 ± 0.01 ^a	0.16 ± 0.02 ^a	0.13 ± 0.01 ^a	0.13 ± 0.01 ^a	0.12 ± 0.01 ^a
Fe	Green	0.14 ± 0.004	0.11 ± 0.02 ^a	0.20 ± 0.02 ^b	0.13 ± 0.02 ^a	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.15 ± 0.01 ^{ab}	0.16 ± 0.01 ^{ab}	0.13 ± 0.01 ^a
	Senesced	0.43 ± 0.025	0.50 ± 0.15 ^b	0.78 ± 0.15 ^c	0.75 ± 0.13 ^c	0.37 ± 0.04 ^{ab}	0.26 ± 0.02 ^a	0.43 ± 0.05 ^{ab}	0.34 ± 0.02 ^{ab}	0.33 ± 0.02 ^{ab}
Mn	Green	0.084 ± 0.007	0.030 ± 0.007 ^a	0.054 ± 0.010 ^a	0.046 ± 0.005 ^a	0.053 ± 0.005 ^a	0.099 ± 0.018 ^{ab}	0.083 ± 0.014 ^{ab}	0.122 ± 0.025 ^b	0.128 ± 0.023 ^b
	Senesced	0.13 ± 0.017	0.098 ± 0.026 ^a	0.108 ± 0.015 ^a	0.095 ± 0.012 ^a	0.085 ± 0.007 ^a	0.135 ± 0.025 ^{ab}	0.123 ± 0.026 ^{ab}	0.211 ± 0.064 ^b	0.104 ± 0.018 ^a
B	Green	0.032 ± 0.001	0.024 ± 0.004 ^b	0.040 ± 0.003 ^c	0.012 ± 0.002 ^a	0.032 ± 0.003 ^{bc}	0.025 ± 0.002 ^b	0.038 ± 0.002 ^c	0.035 ± 0.002 ^{bc}	0.035 ± 0.004 ^{bc}
	Senesced	0.036 ± 0.001	0.033 ± 0.004 ^b	0.042 ± 0.003 ^b	0.012 ± 0.001 ^a	0.037 ± 0.003 ^b	0.029 ± 0.003 ^b	0.041 ± 0.003 ^b	0.038 ± 0.002 ^b	0.037 ± 0.007 ^b
Zn	Green	0.024 ± 0.001	0.021 ± 0.003 ^{ab}	0.036 ± 0.006 ^c	0.024 ± 0.002 ^{abc}	0.019 ± 0.001 ^{ab}	0.024 ± 0.002 ^{abc}	0.017 ± 0.002 ^a	0.030 ± 0.004 ^{bc}	0.016 ± 0.002 ^a
	Senesced	0.057 ± 0.002	0.061 ± 0.008 ^{ab}	0.079 ± 0.006 ^b	0.074 ± 0.005 ^b	0.053 ± 0.003 ^{ab}	0.049 ± 0.005 ^a	0.055 ± 0.004 ^{ab}	0.054 ± 0.004 ^{ab}	0.043 ± 0.006 ^a
Cu	Green	0.013 ± 0.0003	0.012 ± 0.003 ^a	0.015 ± 0.001 ^a	0.013 ± 0.001 ^a	0.012 ± 0.001 ^a	0.015 ± 0.001 ^a	0.012 ± 0.001 ^a	0.012 ± 0.001 ^a	0.014 ± 0.001 ^a
	Senesced	0.014 ± 0.0004	0.014 ± 0.002 ^a	0.017 ± 0.001 ^a	0.015 ± 0.002 ^a	0.013 ± 0.001 ^a	0.012 ± 0.001 ^a	0.013 ± 0.001 ^a	0.014 ± 0.001 ^a	0.012 ± 0.001 ^a
Mo	Green	0.0023 ± 0.0001	0.0023 ± 0.0002 ^a	0.0020 ± 0.0001 ^a	0.0025 ± 0.0002 ^a	0.0022 ± 0.0001 ^a	0.0025 ± 0.0001 ^a	0.0023 ± 0.0001 ^a	0.0023 ± 0.0001 ^a	0.0023 ± 0.0002 ^a
	Senesced	0.0028 ± 0.0001	0.0031 ± 0.0005 ^a	0.0024 ± 0.0003 ^a	0.0029 ± 0.0003 ^a	0.0028 ± 0.0002 ^a	0.0030 ± 0.0002 ^a	0.0028 ± 0.0002 ^a	0.0029 ± 0.0001 ^a	0.0027 ± 0.0003 ^a

Data are mean ± s.e.

Different letters in the same row denote significant differences among plant functional types at $P < 0.05$ n , number of site species.

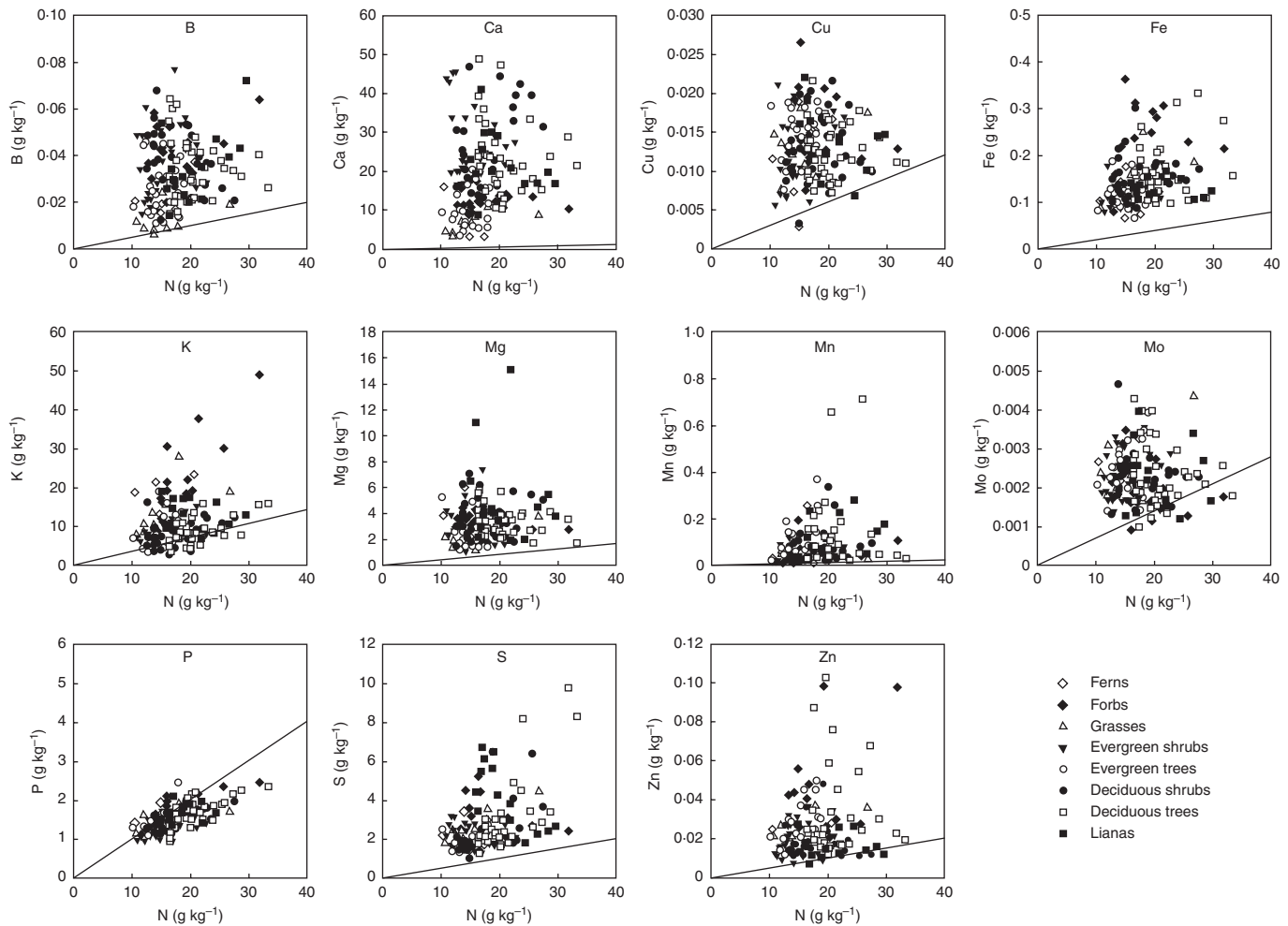


FIG. 1. Concentrations of 11 nutrients (B, Ca, Cu, Fe, K, Mg, Mn, Mo, P, S and Zn) versus N concentrations in green leaves of karst plants of south-western China ($n = 172$). Data are means of site species. The solid lines denote the optimal ratios suggested by Linder (1995).

concentrations of N, P and K in green leaves, respectively. The β value for Mg did not differ from 1, indicating no effect of Mg concentration in green leaves on RE of Mg. Moreover, the effect of $[\text{nutrient}]_{\text{green}}$ on nutrient RE depended on functional type (Table 2). The β value was >1 for N in forbs, evergreen shrubs and trees and for P in forbs, evergreen shrubs and trees, and deciduous trees. For K, all functional types had $\beta > 1$ except for grasses and lianas, while β values for Mg did not significantly differ from 1 in all functional types.

The β values were also estimated for other nutrients (B, Ca, Fe, Mn, S and Zn) that accumulated in senesced leaves. Across all data, for Ca and Zn $\beta < 1$ (Supplementary Data Fig. S1), while β values did not differ from 1 for B, Mn and S. For Fe the β value was >1 , but the correlation between Fe concentrations in green and senesced leaves was very weak ($R^2 = 0.13$). The responses in β estimated for B, Ca, Fe, Mn, S and Zn also depended on functional type (Table 2).

Across all data, the correlations between nutrient RE and the ratios of N to other nutrients in green leaves were not significant (data not shown), while the values of $[\text{nutrient}]_{\text{sen}}$ were significantly correlated with the ratios of N to other nutrients for the 13 nutrients except for P, Cu, Mo and Na (Table 3;

Supplementary Data Fig. S2). The RP of N decreased with increasing N:P ratio in green leaves, while the RPs of K and Mg increased with increasing N:K and N:Mg ratios, respectively, which indicated that the RPs of N, K and Mg increased as the relative limitations of these nutrients increased. Significant correlations for K and Mg were also observed in most functional types, while correlations for N were found in forbs, grasses and deciduous shrubs and for P only in deciduous trees (Table 3).

For B, Ca, Fe, Mn, S and Zn, the values of $[\text{nutrient}]_{\text{sen}}$ were negatively correlated with the N:B, N:Ca, N:Fe, N:Mn, N:S and N:Zn ratios in green leaves, respectively (Supplementary Data Fig. S2), suggesting that concentrations of these nutrients in senesced leaves decreased with their increasing relative limitations compared with N. The correlations for these nutrients also depended on functional type (Table 3).

Nutrient concentrations and resorption patterns among vegetation types

For evergreen shrubs, deciduous shrubs and deciduous trees, differences in nutrient concentrations and resorption among vegetation types were usually not significant, but there were

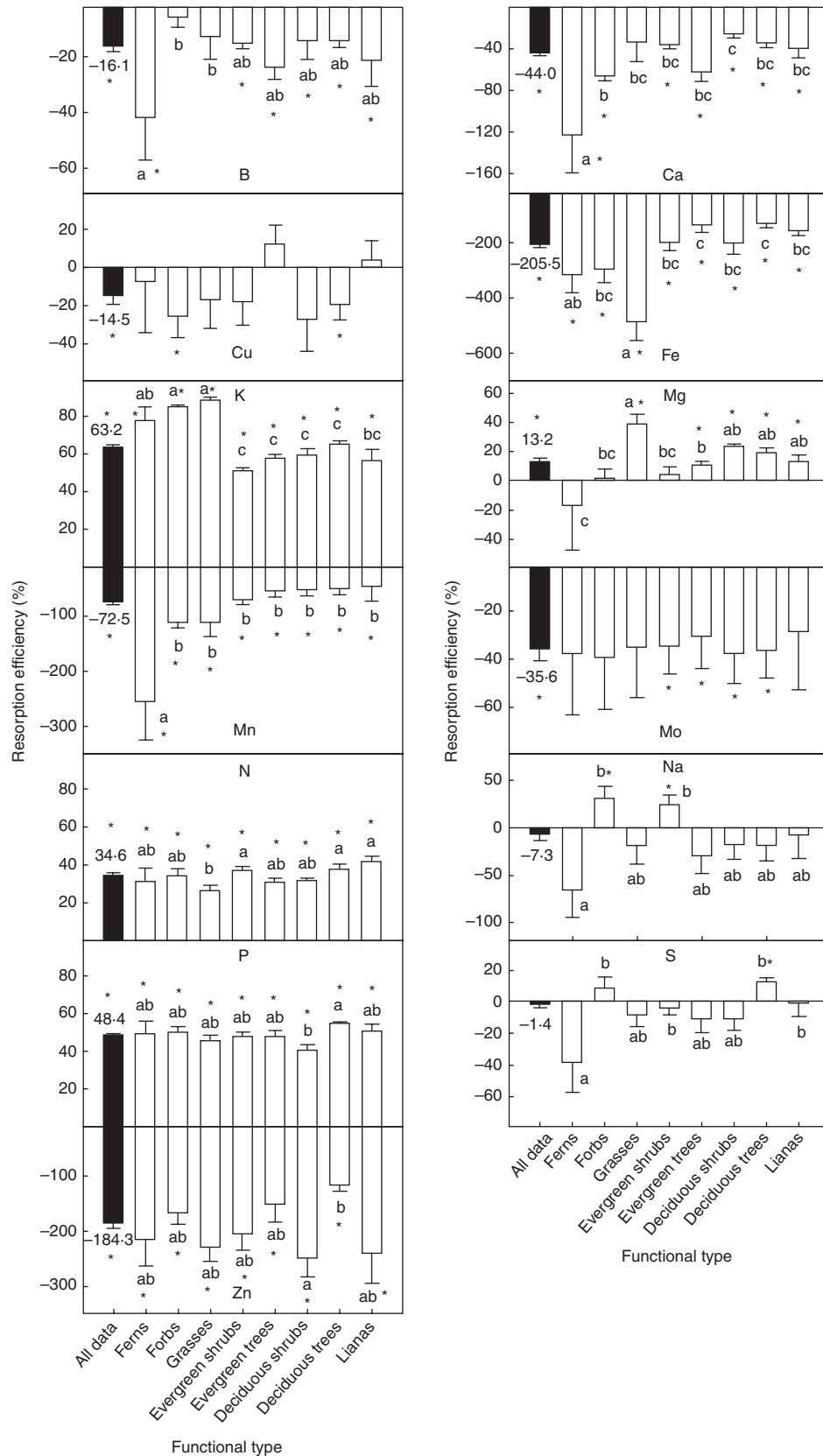


FIG. 2. Resorption efficiencies of 13 nutrients in different plant functional types (mean \pm s.e.). Different letters denote significant differences among different plant functional types at $P < 0.05$. *Significantly different from 0% resorption efficiency at $P < 0.05$.

TABLE 2. β coefficients for different plant functional types

Nutrient	All data		Forbs		Grasses		Evergreen shrubs		Evergreen trees		Deciduous shrubs		Deciduous trees		Lianas	
	R^2	β	R^2	β	R^2	β	R^2	β	R^2	β	R^2	β	R^2	β	R^2	β
B	0.66	0.95	0.44	0.84			0.67	0.93	0.47	0.87	0.51	0.99	0.48	0.78	0.83	1.49*
Ca	0.78	0.85*	0.79	1.85*			0.66	0.69*	0.51	0.66	0.75	0.75*	0.75	0.97	0.64	0.97
Fe	0.13	3.16*	0.44	1.55			0.18	5.08*								
K	0.29	1.86*	0.82	1.81*			0.64	2.07*	0.78	1.61*	0.63	1.84*	0.64	1.46*		
Mg	0.58	1.08	0.29	1.10			0.54	1.26	0.59	0.85	0.60	0.94	0.54	1.2	0.84	0.86
Mn	0.61	0.89	0.41	0.90	0.40	1.20	0.63	0.71*	0.41	0.82	0.71	0.92	0.83	1.09		
N	0.40	1.32*	0.49	1.58*	0.75	1.44	0.38	1.55*	0.68	1.68*	0.47	0.90			0.54	1.34
P	0.25	2.25*	0.62	1.72*	0.60	1.49	0.23	3.26*	0.29	2.92*			0.13	2.29*		
S	0.53	0.91	0.29	0.40*	0.71	0.48*	0.54	1.57*	0.62	2.27*	0.56	1.13	0.56	0.75	0.68	1.15
Zn	0.20	0.63*	0.50	0.54*									0.28	0.47*		

The β coefficients were estimated by fitting eqn (3) using major axis regression.

Data on Cu, Mo and Na were not included because significant correlations between concentrations in green and senesced leaves for these three nutrients were not observed in all plant functional types.

Empty cells denote that there was no significant relationship between nutrient concentrations in green and senesced leaves.

* β is significantly different from 1 ($P < 0.05$), indicating that nutrient resorption efficiency depends on nutrient concentration in green leaves. Ferns were not included due to limited data.

TABLE 3. Summary of Pearson's correlation coefficients (R) relating the ratios of N to other nutrients in green leaves and nutrient concentrations in senesced leaves for different functional types

Nutrient	All data	Forbs	Grasses	Evergreen shrubs	Evergreen trees	Deciduous shrubs	Deciduous trees	Lianas
B	-0.72**	-0.36	-0.08	-0.74**	-0.78**	-0.72**	-0.53**	-0.81**
Ca	-0.81**	-0.60**	-0.12	-0.83**	-0.65**	-0.67**	-0.75**	-0.79**
Fe	-0.31**	-0.64**	-0.67*	-0.22	0.12	0.07	-0.18	0.16
K	-0.40**	-0.77**	0.09	-0.71**	-0.83**	-0.75**	-0.71**	0.23
Mg	-0.64**	-0.28	-0.11	-0.71**	-0.80**	-0.59**	-0.68**	-0.91**
Mn	-0.72**	-0.57*	-0.3	-0.78**	-0.59*	-0.74**	-0.88**	-0.19
N	0.40**	0.64**	0.75**	0.33	0.27	0.68**	-0.15	0.57
P	-0.13	0.08	0.36	-0.14	-0.18	0.11	-0.33*	-0.36
S	-0.65**	-0.47*	-0.54	-0.56**	-0.74**	-0.60**	-0.68**	-0.82**
Zn	-0.49**	-0.62**	-0.61*	-0.53**	-0.23	-0.26	-0.59**	-0.23

Ferns were not included due to limited data.

For N, the relationship between N:P ratio in green leaves and N concentration in senesced leaves was explored.

For Cu, Mo and Na, there were no significant correlations in any plant functional type.

* $P < 0.05$; ** $P < 0.01$.

some exceptions (Supplementary Data Fig. S3). For example, deciduous shrubs showed the lowest Mg concentrations in green and senesced leaves and the lowest RE for Mg in shrublands and the highest values in grasslands. Deciduous trees showed the highest S concentrations and RE for S in grasslands and the lowest values in forests. Evergreen and deciduous shrubs also showed the lowest K concentrations and highest RE for K in grasslands.

Usually, the effects of soil nutrient contents on leaf nutrient concentrations were small, except that B and S concentrations in green leaves of evergreen shrubs and K concentrations in deciduous shrubs were positively correlated with contents of B, S and K in soils ($R^2 = 0.92, 0.95$ and 0.92 for B, S and K, respectively). Evergreen shrubs showed a trend of increasing P ($R^2 = 0.94, P = 0.033$), K ($R^2 = 0.61, P < 0.2$) and Mg ($R^2 = 0.72, P = 0.15$) REs with decreasing soil P, K and Mg contents, respectively. Deciduous trees tended to increase the RE of N as soil N content decreased ($R^2 = 0.84, P = 0.084$). Evergreen shrubs

also revealed significant increases in K ($R^2 = 0.99, P = 0.006$) and Mg ($R^2 = 0.93, P = 0.035$) RPs with decreasing soil Mg and K contents, respectively. Deciduous shrubs tended to increase the RPs of N ($R^2 = 0.78, P = 0.12$) and K ($R^2 = 0.73, P = 0.15$) as soil N and K contents decreased.

The 13 nutrients showed different change patterns among the vegetation types (Fig. 3). For Cu, Mo, Na and P, there was no significant difference in nutrient variables among the four vegetation types. The B, Fe, S and Zn concentrations in senesced leaves differed among the four vegetation types, with grasslands having the highest Fe, S and Zn concentrations and shrublands the highest B concentration. The Ca, K, Mg, Mn and N concentrations in both green and senesced leaves were significantly different among the four vegetation types. Forests had the highest N, Ca and Mn concentrations in green and senesced leaves, while grasslands had the lowest. Shrublands showed the highest K and the lowest Mg concentrations. Grasslands had the highest REs for K and Mg and highest accumulations of Fe, Zn and Mn.

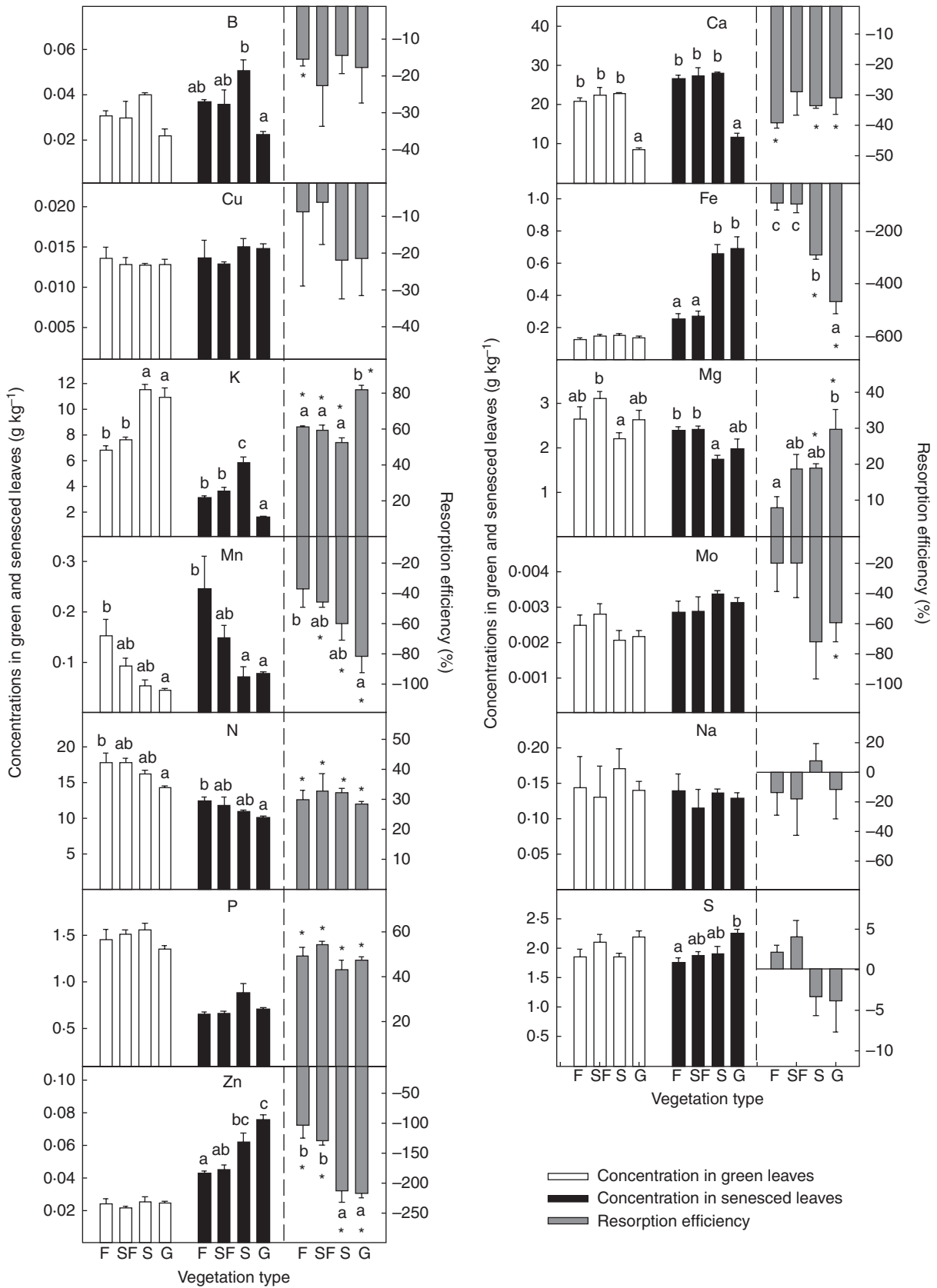


FIG. 3. Nutrient concentrations and resorption efficiencies of the four vegetation types: forest (F), shrub forest (SF), shrubland (S) and grassland (G). Data are means of three sampling sites for each vegetation type. Different letters denote significant differences among vegetation types at $P < 0.05$. *Significantly different from 0% resorption at $P < 0.05$.

DISCUSSION

Nutrient concentrations in karst plants

The values of [nutrient]_{green} of the karst plants in the present study were in accordance with previous observations in this region (Hou, 1982; Zhou, 1997; Yang *et al.*, 2007) and within the normal ranges for healthy plant growth (Marschner, 1995; White and Brown, 2010). The mean concentrations of Fe, K, Mn, N, Na, P and S in the green leaves of karst plants were significantly lower than average values for Chinese plants reported by Han *et al.* (2011). The mean concentrations of Cu, Mn, N, Na and P in green leaves were also lower than global averages (Elser *et al.*, 2000a; Reich and Oleksyn, 2004; Watanabe *et al.*, 2007). The relatively low nutrient concentrations of karst plants could be explained by the shortage of soil nutrients resulting from the shallow soil and the limited total soil mass (Zhang and Wang, 2009; Guo *et al.*, 2011). For example, the average depth of topsoil on karst hills was 2–9 cm, which was much lower than depths in other non-karst habitats (Zhang and Wang, 2009; Liu *et al.*, 2013). Total N and P stocks in the topsoil of forests in the karst region (total N 4.29 t hm⁻², total P 0.24 t hm⁻²; Du *et al.*, 2010) were also markedly lower than those of subtropical evergreen broad-leaved forests in a non-karst region in the same latitudinal zone (total N 12.13 t hm⁻², total P 1.45 t hm⁻²; Yan *et al.*, 2007).

Generally, nutrient concentrations are higher in deciduous than in evergreen species and higher in forbs than in grasses (Aerts, 1996; Han *et al.*, 2011). However, we found that not all the 13 nutrients in the studied karst plants followed such general patterns. For example, the concentrations of Na, Cu and Mo in green and senesced leaves did not differ among functional types (Table 1). Nutrient concentrations differed between forbs and grasses only for Ca, K, Mg, B and Fe. Significant differences in nutrient concentrations between deciduous and evergreen species were observed only for B, Ca, Mn, Mg, N and P. Our results indicated that the previously demonstrated consistent differences were not universal in karst plants, especially for nutrients required in only small amounts.

Relative nutrient limitations to karst plants

The concentrations of Ca, K, Mg, S, Fe, Mn, B, Zn, Cu and Mo in green leaves were mostly higher than the optimal ratios (Fig. 1), indicating that N limitation was dominant compared with these nutrients (Knecht and Göransson, 2004). However, most P concentrations were lower than the optimal ratio, indicating that for most plants P limitation was more important than N limitation. This was confirmed by the mean P concentration in green leaves of either all plants or each functional type being lower than the physiological concentration requirement of P. The K concentrations in shrubs and trees were also lower than the physiological concentration requirement. However, most K:P ratios in karst plants (data not shown) were higher than the optimal K:P (3.5) suggested by Linder (1995). Olde Venterink *et al.* (2003) also suggested that K:P > 3.4 indicated P limitation compared with K. According to the hypothesis of stability of limiting elements (Han *et al.*, 2011), nutrients that are frequently limiting factors would be more stable and less sensitive to environmental gradients. Among the 13 nutrients, the relative variability in green leaves (indicated by CV) was least

for P (21.6 %) followed by N, indicating that P limitation was most important for karst plants. As high N deposition (3.1 g m⁻² year⁻¹) has occurred in this region (Liu *et al.*, 2008), deficiency of plant-available P tends to limit plant productivity in karst habitats due to lowered mobility of P bound to calcium phosphates in calcareous soils (Niinemets and Kull, 2005; Piao *et al.*, 2005; Du *et al.*, 2011).

Nutrient resorption and plant functional types

Most previous studies have focused on N and P resorption, ignoring other nutrients that are important for modelling nutrient cycling and ecosystem productivity (Han *et al.*, 2011; Vergutz *et al.*, 2012). We reported the resorption patterns of 13 nutrients in karst plants. The average RE of N (34.6 %) was lower than the global averages (46.9–50 %; *t*-test, *P* < 0.05), while the average RE of P (48.4 %) was close to the global values (52–53.5 %) reported by Aerts (1996) and Yuan and Chen (2009a). The mean REs of K (63.2 %) and Mg (13.2 %) were lower than the global averages (70.1 and 28.6 %, respectively) calculated by Vergutz *et al.* (2012), who introduced a mass loss correction factor into resorption estimates to overcome the error resulting from leaf mass loss during senescence. In contrast to the global average RE of Ca (10.9 %) (Vergutz *et al.*, 2012), Ca accumulated in senesced leaves of karst plants. Previous studies suggested that, as a structural element in plants, Ca is usually conserved in leaves (Lambers *et al.*, 1998; van Heerwaarden *et al.*, 2003). Moreover, the plot of Ca versus N (Fig. 1) suggested that excessive uptake of Ca generally occurred in karst plants on calcareous soils. A previous study reported significant REs of S, Fe, Cu and Mn in deciduous trees (Hagen-Thorn *et al.*, 2006). In our study, only a very low RE of S (12.5 %) was observed in deciduous trees, and Fe, Cu and Mn showed accumulations in senesced leaves (Fig. 2). It seemed that most nutrients that were required in only small amounts were excessively taken up by plants (Fig. 1) and retained in leaves.

Differences in RE among plant functional types have well been documented (Aerts, 1996; Yuan and Chen, 2009a; Vergutz *et al.*, 2012). In the present study, the differences in the REs of N and P among functional types were small, consistent with the results of Aerts (1996), while large differences in the REs of K and Mg were observed (Fig. 2). Large differences in accumulations of B, Ca, Fe and Mn among functional types were also observed (Fig. 2). Similar patterns of REs for K and Mg among functional types were reported by Vergutz *et al.* (2012). Because nutrient RE depends on the transfer of nutrients between leaf and other plant pools, the size of the non-leaf nutrient pools could greatly affect nutrient resorption (Kull and Kruijt, 1999; Vergutz *et al.*, 2012). The smaller non-leaf pools in grasses could lead to a greater need for nutrient resorption compared with other functional types, such as shrubs and trees (Vergutz *et al.*, 2012).

Nutrient resorption could play an important role in regulating ecosystem nutrient cycling, mainly affecting litter qualities that control decomposition rates (Aerts, 1997; Reed *et al.*, 2012). In the karst region, different nutrient resorption patterns among plant functional types greatly affected litter nutrient concentrations. For example, grasses with higher REs for K and Mg produced litter with lower K and Mg concentrations than deciduous shrubs and trees (Fig. 2; Table 1). Residues with poor levels of nutrients release these nutrients more slowly than those with

high levels (Parton *et al.*, 2007). Thus, the changes in relative compositions of different plant functional types during vegetation degradation would significantly influence nutrient cycling in karst ecosystems. The return of nutrients from vegetation pools to soil pools was slower in shrublands and grasslands, whose dominant species (e.g. evergreen shrubs and grasses) usually had low litter nutrient concentrations (Table 1).

The mean RPs of N (1.13 %) and P (0.077 %) of the karst plants were lower than the global averages of N (0.87–1.0 %) (Killingbeck, 1996; Yuan and Chen, 2009b; Vergutz *et al.*, 2012) and P (0.06 %) (Killingbeck, 1996); while mean values of K (0.38 %) and Mg (0.27 %) were higher than the respective global averages of 0.47 and 0.35 % reported by Vergutz *et al.* (2012) (*t*-test, $P < 0.05$). Killingbeck (1996) suggested that N concentrations in senesced leaves of <0.7 % and P concentrations <0.04 – 0.05 % could be considered as ‘complete resorption’. According to this criterion, most of the plant functional types did not resorb N and P completely (Table 1). Differences in RP tended to be larger than those in RE among functional types. For instance, the RPs of N and P were higher in evergreen than in deciduous shrubs (Table 1), whereas the REs of N and P did not differ between the two functional types (Fig. 2). The RP of K was higher in grasses than in forbs, whereas the RE of K did not differ. These results confirmed that nutrient RP appeared to be a more definitive and objective measure of the degree to which selection has acted to minimize nutrient loss (Killingbeck, 1996; Wright and Westoby, 2003).

Nutrient resorption and nutrient status

The relationships between nutrient resorption and plant nutrient status are still being debated. In the present study, across all data, the REs of N, P and K increased with their decreasing concentrations in green leaves ($\beta > 1$). The RE of Mg was not controlled by Mg concentration in green leaves ($\beta = 1$; Supplementary Data Fig. S1), which was in contrast to $\beta > 1$ estimated for Mg from a global data set (Vergutz *et al.*, 2012). Nutrient resorption reflects the trade-off of the energy cost for plants between taking up nutrients from soil and resorbing nutrients from senescent leaves (Wright and Westoby, 2003; Jiang *et al.*, 2012). The value of RE is expected to increase with lowered [nutrient]_{green} ($\beta > 1$), if taking up nutrients from soil with poor nutrient conditions is more expensive for plants than resorbing them from senescent leaves (Wright and Westoby, 2003; Kobe *et al.*, 2005; Kazakou *et al.*, 2007). In the karst region, soil developed on dolomite or limestone rock is usually rich in Mg (Zhang and Wang, 2009). Plants can easily acquire Mg from soil, which could explain why the β value for Mg did not differ from 1 (Table 2). For Ca and Zn, which accumulated in senesced leaves, $\beta < 1$, suggesting that accumulations of Ca and Zn decreased with their increasing concentrations in green leaves. When data were grouped by functional type, the effect of [nutrient]_{green} on nutrient RE seemed to disappear for many functional types (Table 2); similar results were observed in a global data set (Vergutz *et al.*, 2012). It was likely that a small range of [nutrients]_{green} and small sample size in a single functional type failed to reveal this effect (Kobe *et al.*, 2005), as nutrient concentrations differed among functional types in the present study (Table 1).

Unexpectedly, we found no relationship between nutrient REs and the ratios of N to other nutrients in green leaves, suggesting

that the relative nutrient limitations indicated by the ratios of N to other nutrients were poor predictors of nutrient RE. In a savanna ecosystem, foliar N:P ratios also did not predict the REs of N and P (Ratnam *et al.*, 2008), whereas the N:P ratio was a good indicator for the RE of P in a P-limited wetland (Rejmánková, 2005). In contrast, the RPs of N, K and Mg were indicated by the N:P, N:K and N:Mg ratios, respectively (Table 3; Supplementary Data Fig. S2), suggesting that relative nutrient limitations could predict RPs of N, K and Mg in the karst habitats, in accordance with previous observations in other ecosystems (Rejmánková, 2005; Ratnam *et al.*, 2008). In addition, for nutrients (B, Ca, Fe, Mn, S and Zn) that accumulated in senesced leaves, their concentrations in senesced leaves increased with their lowered relative limitations compared with N.

In contrast to our expectation, vegetation degradation did not decrease most soil nutrient contents except for K, and differences in soil nutrients among vegetation types were usually small (Supplementary Data Table S1). However, effects of soil nutrients on plant nutrient resorption were detected, especially for evergreen shrubs, which tended to increase the REs of P, K and Mg and RPs of K and Mg as soil P, K and Mg contents decreased. Evergreen shrubs (e.g. *I. yunnanensis*, *P. fortuneana* and *Rh. heterophylla*) usually dominate in the karst soils that have low nutrient availabilities, which could be explained by their enhanced nutrient reuse from senesced leaves and lowered nutrient requirements from soil.

Nutrient concentrations and resorption patterns among vegetation types

Nutrient concentrations of the same functional types usually showed no significant differences among the four vegetation types (Supplementary Data Fig. S3), which may be due to the small variability in most soil nutrients (Supplementary Data Table S1). The remarkably low K content in the soil of grasslands probably caused the lowest K concentrations of evergreen and deciduous shrubs in this vegetation type. Consequently, we attributed the significant differences in nutrient concentrations of different vegetation types to the different species compositions rather than soil conditions. For example, grasslands showed the highest K concentration in green leaves and RE for K (Fig. 3), because the dominant functional types (grasses and forbs) had higher K concentrations (Table 1) and REs for K (Fig. 2).

Conclusions

According to the stoichiometry of 13 nutrients in green leaves, plant growth was mainly limited by P in the karst region of China. However, further fertilization experiments should be conducted to test this. Overall, four nutrients (N, P, K and Mg) revealed resorption, seven (B, Ca, Cu, Fe, Mn, Mo and Zn) accumulated in senesced leaves and two (Na and S) did not show resorption or accumulation. The RE of N, P and K and the accumulation of Ca and Zn increased with their decreasing concentrations in green leaves. The N:P, N:K and N:Mg ratios in green leaves predicted the RPs of N, K and Mg, respectively. As different nutrient resorption patterns among plant functional types significantly affected litter qualities, further studies are needed to reveal how litter nutrient concentrations regulate the process of

decomposition and to improve our understanding of nutrient cycling in karst ecosystems.

SUPPLEMENTARY INFORMATION

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: nutrient contents of topsoil of the four vegetation types. Table S2: detailed information of the sampling sites of the four vegetation types. Table S3: list of the studied plant species in the four vegetation types. Figure S1: relationships between nutrient concentrations in green and senesced leaves. Figure S2: relationships between the ratios of N to other nutrients in green leaves and nutrient concentrations in senesced leaves. Figure S3: nutrient concentrations and resorption efficiencies of evergreen shrubs, deciduous shrubs and deciduous trees in the four vegetation types.

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