Trees
https://doi.org/10.1007/s00468-021-02096-w

ORIGINAL ARTICLE

Plantations modified leaf elemental stoichiometry compared to the native shrub community in karst areas, Southwest of China

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Received: 4 June 2020 / Accepted: 22 January 2021

Abstract

Key message **Nutrient limitation changed from N–P co-limitation in the native shrubs to N limitation in the plantations.**

Abstract Element stoichiometry is a powerful tool to examine plant–soil nutrient feedbacks. In karst ecosystems, southwest China, afforestation has been widely adopted to restore soil fertility and enhance ecosystem functioning under degraded native shrub stands. However, it is unclear whether and how multiple-element stoichiometry of plants in afforested forests would differ from the native shrub community. We investigated the concentrations of C, N, S, P, K, Ca, Mg, Na, Fe, Al, Cu, Zn, and Mn in leaves and soils in native shrub community and three plantations (*Pinus yunnanensis*, *Alnus japonica,* and *Platycladus orientalis*). We found (1) There was significant discrimination in leaf elemental compositions between native shrub community and plantations in the karst region, southwest China. Native shrubs had lower leaf N, P, S and higher C, Ca, and Mg concentrations, as well as C:P and N:P ratios, compared to plantations; (2) For different plant species, grasses had higher P, K, and Na and lower C:P and N:K, compared to trees and shrubs; (3) N:P, K and S concentrations differed most between the native shrubs and plantations; (4) N:P in native shrubs was close to 12 while decreased to 11.3, 10.2 and 9.7 in three plantations. These results suggest that plantations strongly changed the elemental stoichiometry of native shrub communities in the karst region. N:P, Ca:Mg, K and S are key indicators for plant nutrient status in the study area. P limitation alleviates in plantations compared to native shrubs. Our study could be used to guide reforestation and improve ecosystem functioning in the karst region, Southwest China.

Keywords Karst ecosystem · Plantation forest · Native shrub · Multiple elements · Stoichiometry · Nutrient limitation

Introduction

Karst ecosystems, characterized by poor soil, discontinuous soil layers, water shortages, and specialized regional vegetation, cover 10–15% of the ice-free continental surface (Geekiyanage et al. 2019). Under frequent human disturbance, such as urban intensification, agricultural extension, livestock grazing, and firewood production, karst ecosystems are fragile and companied with rocky desertification. In these rocky desertification areas, different rates of vegetation degradation occur due to resource overexploitation including intensive mining, deforestation, and inappropriate agricultural practices (Du et al. 2011; Qi et al. 2013). The accelerated vegetation degradation further aggravated drought and soil infertility in karst ecosystems (LeGrand 1973). To reduce rocky desertification rate and improve karst ecosystems, many re-vegetation policies, such as

Communicated by Lee Kalcsits.

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Published online: 15 February 2021

replenishing plantation forests, have been adopted. Some previous studies reported that different plantations have various potentials for C fixation capacity (Zhang et al. 2017) and alter bacterial community structure (Xue et al. 2017). While other studies found that pure plantations have a negative impact on soil quality (Lu et al. 2014), different plantations may have different C:N:P stoichiometry (Pang et al. 2018). Although these studies provide the theoretical basis for karst vegetation restoration, there is still a knowledge gap on the changes in leaf element stoichiometry between natural/secondary shrubs and plantations and variations among different plantations.

Typically, plants require multiple elements for their growth and development (Mengel et al. 1982). C, N and P are essential nutrients to support plant structure and function. Especially in tropical/subtropical karst areas, P is more deficient than other karst areas which alter the coupling relationships of C, N and P. Ca and Mg are characteristic elements in karst ecosystems because major types of karst-forming rocks contain minerals, such as dolomite ($CaMg(CO_3)_2$) and calcite (Ford and Williams 2013). K and Na are associated with water holding capacity and are considered important indicators for drought (Umair et al. 2020). Previous studies reported various C:N:P ratios across plantations (Pang et al. 2018) and plant functional groups (Pan et al. 2015), which indicated nutrient limitation and nutrient requirement for different plants. However, few studies have been conducted on how multiple-element stoichiometry varies between different plant groups. Multiple-element stoichiometry can reveal some special element characters under specific habitats or requirements (Townsend et al. 2011; Wen et al. 2018; Huang and Li 2019). Therefore, it is important to study plant nutrient uptake and their correlations in karst ecosystems under the frame of multiple-element stoichiometry.

Shrub communities degraded from natural forests are widely distributed in karst regions, Yunnan province, southwestern China, due to human disturbance activities in history. These shrub communities possess low ecosystem services, particularly timber production and water conservation. Across these areas, patched plantation stands have been established during the past decades with multiple purposes. Major tree species in plantations include pine (*Pinus yunnanensis*), cypress (*Platycladus orientalis*) and alder (*Alnus japonica*), which can grow in poor soils with strong drought-resistant ability or/and nitrogen-fixation capacity. How these plantation communities grow and how these different tree species interact with nutrient availability of soils are unclear. The aim of this research is to understand how multiple-element stoichiometry varies between the secondary shrubs and three plantations. We hypothesized that: (1) there were significant differences in element compositions of plants at the community level, among native shrubs and three plantation stands, (2) nutrient limitation could vary between native shrubs and three plantations, and (3) the interactions between chemical properties of soils and plants are modified by plantation trees compared to native shrub communities. The findings generated in this study would provide a theoretical basis for vegetation restoration in karst rocky desertification regions.

Materials and methods

Study area

Our study area is located at Luxi County, Yunnan Province, southwestern China. Mean annual precipitation is 979 mm and temperature is 15.2 °C (1981–2010). Extreme maximum and the lowest temperatures are 34.1 and 7 °C, respectively (1981–2010). Annual average sunshine time is 2122 h. The main soil group is ferralosols according to World Reference Base for Soil Resources (WRB 2014). The zonal vegetation is semi-evergreen broadleaf forests including soapberries (*Sapindus delavayi)*, oak (*Quercus cocciferoides)*, and albizia (*Albizia bracteate)*. These original natural forests have been mostly destroyed due to long-term human activities (cutting, farming, grazing, etc.) in the study area while drought-resistant, secondary shrub communities are the most popular vegetation type. To improve soil quality and restore vegetation, massive plantation stands of different tree species have been established in the past three decades.

In this study, we selected three typical plantation communities including Yunnan pine stand (*P. yunnanensis,* PY), alders stand (*A. japonica,* AJ) and cypresses stand (*P. orientalis,* PO), as well as one native shrub community (SH) from Luxi county afforestation area (Fig. 1 and Table 1). At each site, three 20 m × 20 m plots were set up. The diameter at breast height (DBH) and height of trees were measured and the density of each stand was calculated (Table 1). There were more species in the SH and PY while fewer species in the AJ and PO. Specifically, the SH included 20 species from 12 family; the PY included 17 species from 13 family; the AJ included 10 species from 7 family; and the PO included 7 species from 6 family. Species details are listed in Table S1. The SH had higher coverage excluding bare rock areas. The PY had various species and low coverage, along with exposed litterfall layer. The AJ had higher coverage. The PO was characterized by low coverage, few species, with exposed and compacted soils (Fig. 1). No inorganic fertilizers were used in three plantations. The age of plantations in the current study varies from 15 to 23, which could influence element stoichiometry, however, we have to ignore this factor.

Fig. 1 Photographs of four communities (**a** Native shrub community; **b** Pine community; **c** Alder community; **d** Cypress community). Photo taken by Huimin Tao, in 2017

Table 1 Geographical location of study sites and growth status of plantation trees in Luxi, Yunnan province, China

Communities	Latitude	Longitude	Altitude (m)	Age of plantations	DBH (cm)	Height (m)	Density (stem/hm^2)
Shrubs (SH)	24.5086°N	103.8519°E	2300		–	–	–
P. yunnanensis (PY)	24.5081°N	103.8514°E	2410	18	5.28 ± 0.17	9.87 ± 0.12	5033
A. japonica (AJ)	24.5075°N	103.8506°E	2296	15	3.44 ± 0.15	6.98 ± 0.14	2400
P. orientalis (PO)	24.5297°N	103.8072°E	2006	23	6.31 ± 0.17	15.50 ± 0.05	2500

Sampling

At each sampling site, a total of 7 soil samples (0–10 cm depth) were randomly collected. Roots and debris were removed from the soil samples. We collected leaves from grasses, shrubs, and trees in the four communities. Leaf samples were collected in the upper middle part of the south-facing crown of trees, shrubs, and grasses, and fully expanded medium-sized leaves were selected. For each species at each site, three to five samples were randomly collected from different healthy individuals and then mixed into one sample. Since age may influence nutrient concentrations in evergreen trees (Aerts 1995; Doust 2010), we only used current year leaves for all species in this study. All 28 soil samples and 110 plant samples were collected in summer, 2017.

Chemical analysis

We stored all the samples in coolers and transported them back to the laboratory. Following the removal of mineral particulates on the leaf surfaces with wet absorbent cotton, we dried all leaves at 105 °C for 30 min and 60 °C for 48 h in a forced-draft oven (Markert 1994). We air-dried soil and rock samples for approximately 5 weeks to constant weight. After drying, we ground and homogenized samples using a stainless-steel grinder and then sieved each sample through a 60-inch mesh sieve for total elements concentration analysis and by hands through a 10-inch mesh sieve for available and exchangeable elements concentration and other chemical properties analysis for plants and soils.

Soil pH was determined using a 1:5 soil:water solution (Porter et al. 1987). Soil and leaf samples were digested with trace metal-grade nitric acid and diluted with deionized water (Dahlquist and Knoll 1978). We examined total C, N and S using an elemental analyzer (Vario EL cube; Elementar, Germany) and the concentrations of P, K, Ca, Mg, Mn, Fe, Zn, Cu, Na, and Al, as well as available P, Mn, Fe, Zn, Cu and exchangeable K, Ca, Mg, Na and Al with a plasma optical emission spectrometer (ICP-OES) (Iris Advantage 1000; Thermo Jarrell Ash, Franklin, MA). We measured soil NH_4^{+}-N and NO_3^{-}-N using an Automated Discrete Analyzer (SmartChem 200; Alliance, France).

Statistical analysis

Community weighted means (CWM) represents a community-aggregated value based on community composition, which can be used to explore the variation of functional traits and their causes and consequences for higher-level processes across ecosystems. It can be an important tool to evaluate element concentrations and their ratios differences between communities and plant growth forms considering community composition. Therefore, the community weighted means of stoichiometry has been used to determine multiple elemental stoichiometry (Jiang 2018) and nutrient limitation during community succession based on the importance of each species (Zhang 2015).

CWM was calculated in the following equations:

$$IV_{ij} = \frac{Relativefrequency + Relativedensityorheight + Relativedominanceorcover}{3} \quad (1)$$

$$IV = \sum_{i=1}^{n} IV_{ij} \quad (2)$$

$$CWM_k = \frac{\sum_{i=1}^{n} C_{ijk} \times IV_{ij}}{IV} \quad (3)$$

where IV_{ij} is the importance value of species i in plot j. IV is the sum of IV_{ij} in plot j, n is the number of species in the plot. C_{ijk} is the element k concentration of species i in plot j. CWM_k represented the community weighted means of element k. IV were calculated for each species (Table S2).

The analysis of variance (ANOVA) was conducted to test the difference of element concentrations and their ratios among four plant communities and three plant growth forms. To determine whether the multiple elemental concentrations and ratios in plants could discriminate between communities and between growth forms, and to identify what elements and ratios had higher discriminatory power, we performed Canonical Discriminant Analysis (CDA) (Karimi and Folt 2006). To calculate the correlations between leaf and soil elements, we conducted bivariate correlation analysis. Correlations between elements were analyzed using Pearson correlation tests. ANOVA and CDA were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). All data were $\log_{10}$ transformed to homogenize and normalize distributions prior to analysis when necessary. DataGraph 4.6 (Visual Data Tools, Inc) was used for drawing.

Results

Differences in elemental compositions between communities

Plant elemental compositions were significantly discriminated among four communities based on CDA (Fig. 2). The SH was significantly separated from other three plantation communities by the canonical 1 while the PY was significantly separated from other three plantation communities by the canonical 2. The first two functions in the CDA explained a total of 91.4% of the variance (Table S3). Particularly, P, S, C and Mg concentrations contributed the most to the difference between the SH and other three communities (High absolute values of coefficients, Table S4). And N, C, Al and Mn concentrations

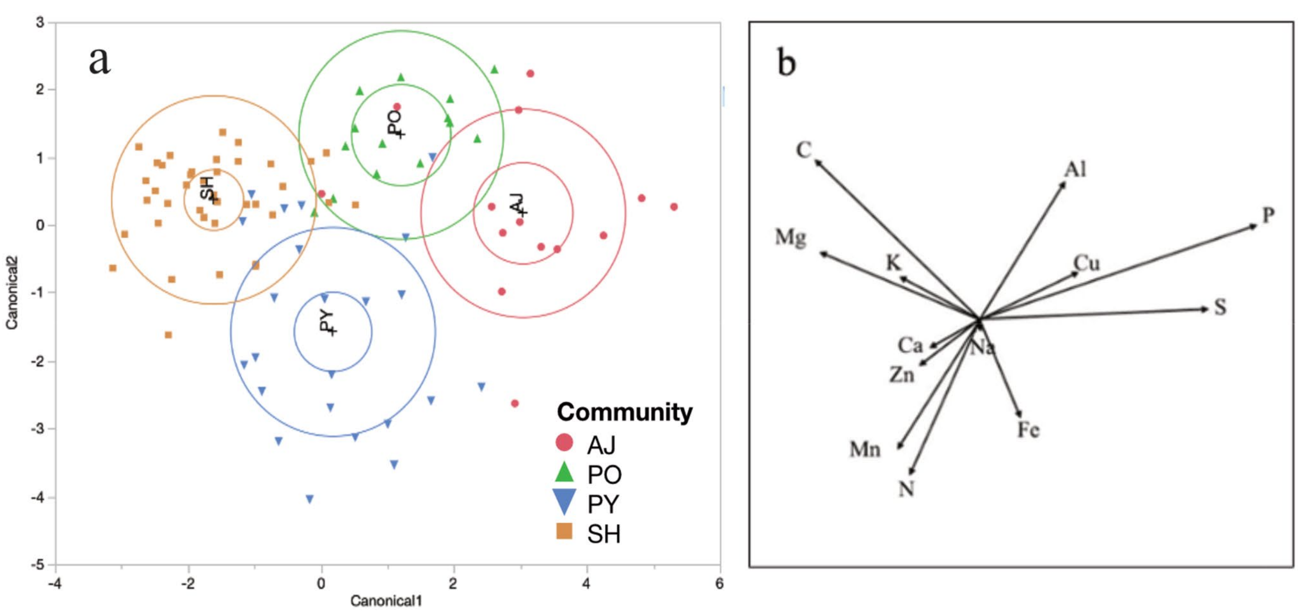

Fig. 2 Four communities defined by the first two functions (Canonical 1 and Canonical 2) extracted from the Canonical Discriminant Analysis (CDA) based on leaf element composition. SH refers to shrub community, PY refers to Yunnan pine community, AJ refers to alder community and PO refers to cypress community. Arrows of original variables equals to their canonical coefficients and indicate their contributions in differentiating four communities

contribute the most to the difference between the PY and other three communities (Fig. 2, Table S3, S4 and S5).

For four plant communities, Community weighted means of N, P and S was lower at the SH than at least one plantation community while C, Ca and Mg concentrations were higher at the SH. There were higher N, P, S concentrations in the AJ and lower Ca and Mg in PY and AJ (Fig. 3).

For different plant growth forms, there were no significant differences of N concentration among grasses, shrubs, and trees in four communities, but P concentration of grasses was higher than in trees in all plantations. There were lower S and P concentrations of shrubs at the SH than other shrubs at AJ and PO. Grasses had higher P, K, Na concentrations than trees and shrubs at most communities. In native shrub community, CWM of C, P, K, Mg, Zn, Na and Al differed between grasses and shrubs (Fig. 4).

In sum, the leaf C, N and P concentrations in our study area were 462.74 mg/g, 22.48 mg/g and 2.05 mg/g (Table S7).

Characteristics of elemental stoichiometry in different communities

The ratios of C, N and P, as well as the ratio of Ca and Mg were different among the four communities (Table 2). C:N was the highest in the PO (26.18) while the lowest in the AJ (16.14). C:P in the SH was significantly higher than all plantations while N:P in the SH was significantly higher than in the AJ. In addition, Ca:Mg ratio was higher in the PO than PY and AJ.

Specifically, in the SH, there were significantly higher C:N, C:P, N:P, N:K, and Ca:Mg ratios as well as lower K:P in shrubs than grasses. There were no difference of C:N in three plant growth forms in each community. C:P of trees was higher than grasses in all three plantations (Table 3). For N:P, trees had higher ratio values than shrubs and grasses in the PY and PO. There were significant differences of Ca:Mg between shrubs and grasses in the shrubs and between trees and shrubs and grasses in the PO. Trees had the lowest K:P in all plantations while grasses had the lowest N:K in PY and PO (Table 3).

For trees, Ca:Mg was higher in the PO than in other two plantations. K:P in PY was lower than others. For shrubs, C:P, K:P and N:P in the SH were higher than in the AJ or PO. For grasses, C:N was higher in the PO than in the AJ and PY while C:P was the highest in the shrubs than the other three plantations. In addition, N:P of grasses was higher in the shrubs than in the PO.

Correlations between element concentrations in plant leaves in different communities

In our study area, P was positively associated with K and Mg (Table S6). There were stronger correlations between leaf elements in the native shrubs than in the plantations (Fig. 5) but weaker correlations of elements between plants and soil in the native shrubs than in the plantations (Fig. 6). For instance, no significant correlations were found between major elements in native shrubs, however, in plantations, plant P was associated with soil N or P.

In native shrub community, N:P was negatively linked to P while in plantations, N:P was positively linked to N and negatively linked to P (Fig. S1). N:P of trees in plantations was only associated with N (Fig. S2). N:P of shrubs was associated with N and P in all communities (Fig. S3). N:P of grasses was negatively linked to P in native shrubs while being linked to N and P in plantations (Fig. S4).

Soil properties

There were no significant differences of total N, P, S, Ca, Cu and Zn concentrations in soil between 4 communities (Table S8). Ammonium was the highest in AJ while nitrate in SH (Table S9). Both total and exchangeable K were higher in AJ. The ratios of C:N and C:K in SH were lower than PO or PY, respectively (Table S10).

Discussion

Differences of plant stoichiometric compositions between shrub community and plantation stands

Our results clearly showed that plantation establishment altered the ionome and stoichiometry of plants at community level and growth form level compared to the native shrub communities in the karst regions, southern China. For instance, there was a significant separation between shrubs and plantations as well as between PY and other plantations based on CDA (Fig. 2, Tables S3, S4 and S5). Stronger correlations in elemental concentrations between plants and soils in plantation stands were observed compared to the native shrub communities (Fig. 6). These results are consistent with the alternations of elemental compositions in soils when shrub communities were converted to these plantation stands in this area (Tao et al. 2018, 2019). This suggests that afforestation not only changes the compositions of community plants, but also substantially alters the biogeochemistry of ecosystems. In particular, alder stands showed more significance as N fixer and broadleaf tree.

CWM of N, P concentrations and N:P ratios of grasses in our study were higher than another study except in the PO (lower N:P) (Pan et al. 2015). N:P ratios of shrubs and trees in all communities were lower than in Pan's study (about 17) (Pan et al. 2015).

In general, the C concentration (462.74 mg/g) was slightly lower than the global terrestrial plant level of 464 mg/g

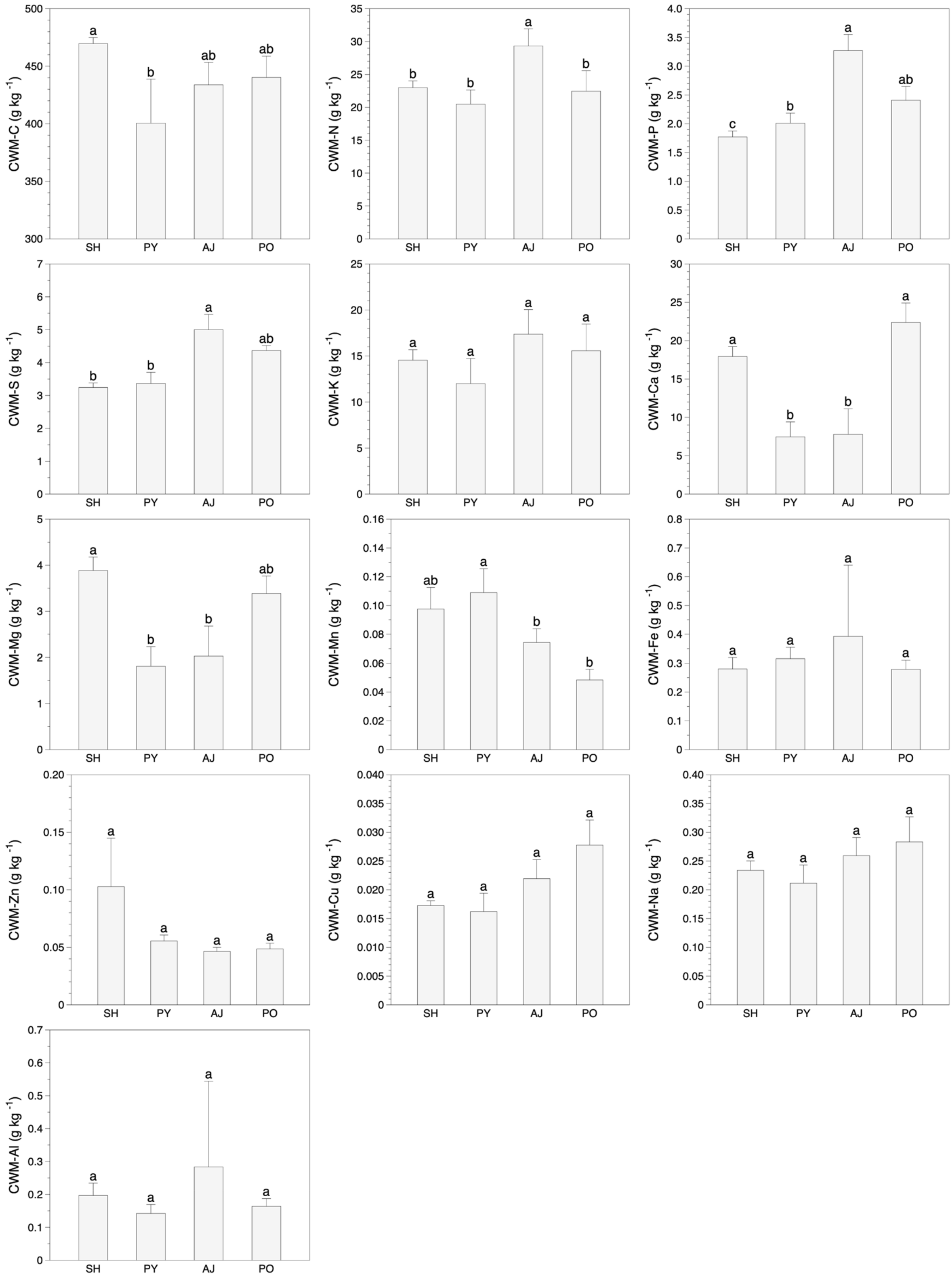

Fig. 3 Community weighted means of leaf element concentrations (mean ± se) in four plant communities. SH represents the shrub community, PY stands for Yunnan pine community, AJ stands for alder community and PO stands for cypress community. Different letters at the top of columns denote significant differences of four communities at 0.05 significant level

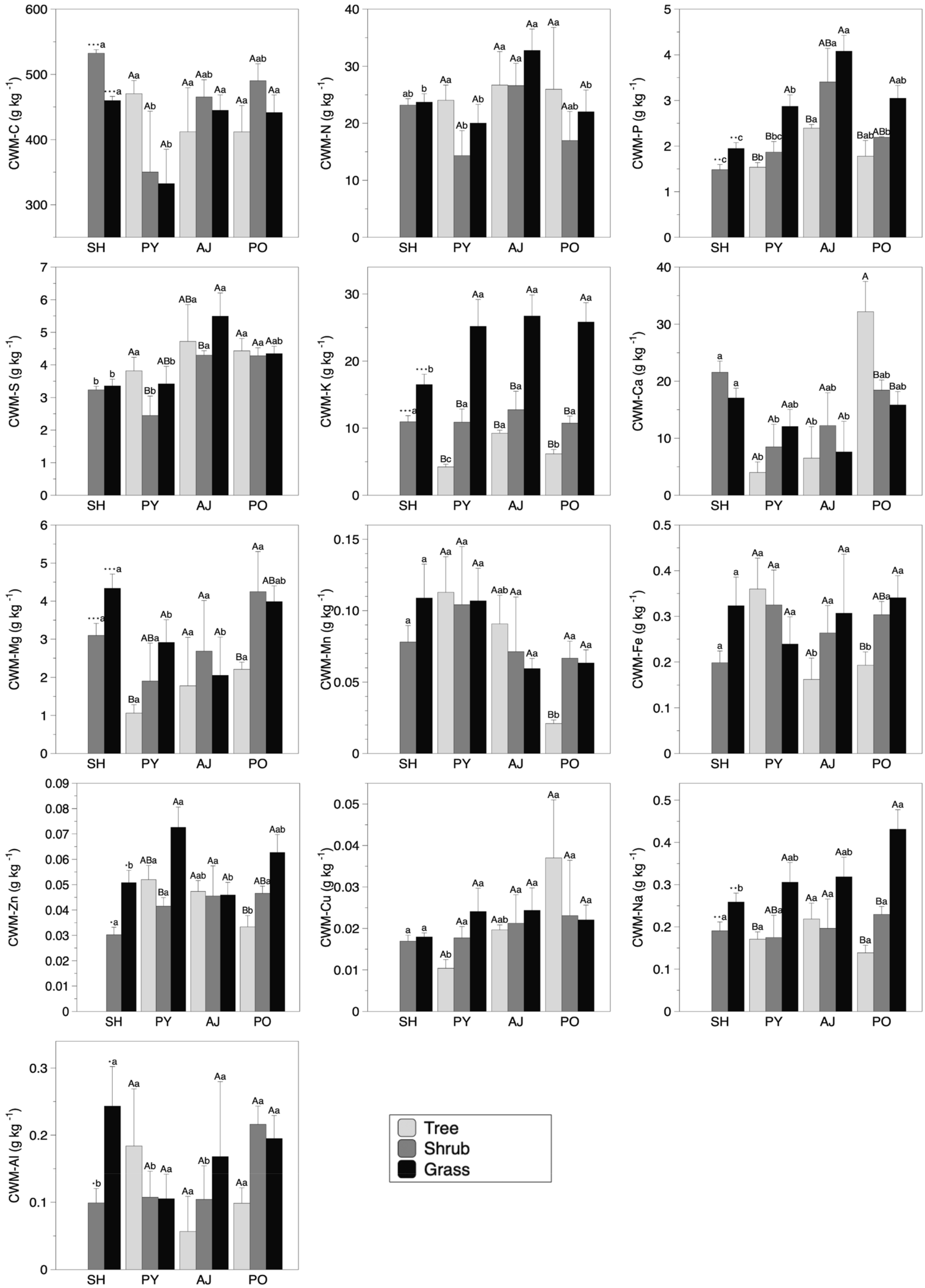

Fig. 4 Community weighted means of leaf element concentrations (mean ± se) of three growth forms between four communities (SH refers to shrub community, PY refers to Yunnan pine community, AJ refers to alder community and PO refers to cypress community). Lowercase letters at the top of columns denote differences of four communities while uppercase letters denote the difference between three plant growth forms at 0.05 significant level (Star denote significant differences of shrubs and grasses in SH. *$p < 0.05$, **$p < 0.01$ ***$P < 0.001$)

Table 2 CWM ratios of elements in plant leaves among four communities. SH refers to shrub community, PY refers to Yunnan pine community, AJ refers to alder community and PO refers to cypress community. Different letters denote significant differences among four communities ($p < 0.05$)

Ratios	SH	Plantations		
		PY	AJ	PO
C:N	21.86 ± 0.9 ab	18.22 ± 1.67 c	16.14 ± 1.36 bc	26.18 ± 3.97 a
C:P	257.12 ± 14.17 a	230.26 ± 25.07 b	145.22 ± 14.89 b	199.33 ± 16.1 b
N:P	11.92 ± 0.53 a	11.28 ± 1.19 ab	9.72 ± 1.18 b	10.15 ± 1.47 ab
Ca:Mg	4.45 ± 0.43 ab	3.27 ± 0.32 b	2.95 ± 0.51 b	7.89 ± 1.27 a
K:P	7.26 ± 0.33 a	5.19 ± 0.85 b	5.05 ± 0.6 b	5.98 ± 0.66 b
N:K	1.74 ± 0.12 b	3.31 ± 0.42 a	2.18 ± 0.33 b	2.29 ± 0.51 b

Table 3 CWM ratios of elements of plant leaves among different communities (Mean ± SE), (SH refers to shrub community, PY refers to Yunnan pine community, AJ refers to alder community and PO refers to cypress community)

Growth forms	Ratios	SH	Plantations		
			PY	AJ	PO
Trees	C:N	–	20.82 ± 2.97 Aa	16.23 ± 2.25 Aa	23.43 ± 10.7 Aa
	C:P	–	315.32 ± 35.45 Aa	172.67 ± 28.34 Ab	245.76 ± 39.22 Aab
	N:P	–	15.53 ± 0.97 Aa	11.31 ± 2.78 Aa	14.79 ± 4.72 Aa
	Ca:Mg	–	3.03 ± 0.96 Ab	2.78 ± 0.7 Ab	14.47 ± 1.66 Aa
	K:P	–	2.76 ± 0.2 Bb	3.86 ± 0.06 Ba	3.64 ± 0.46 Ba
	N:K	–	5.82 ± 0.44 Aa	2.95 ± 0.76 Aa	4.29 ± 1.68 Aa
Shrubs	C:N	26.23 ± 1.3 *a	18.11 ± 4.06 Aa	18.25 ± 2.98 Aa	33.69 ± 9.72 Aa
	C:P	332.85 ± 18.32 ***a	200.16 ± 57.42 ABb	149.39 ± 29.29 Ab	223.43 ± 10.79 Aab
	N:P	13.82 ± 0.87 *a	8.2 ± 2.75 Bab	8.8 ± 2.36 Aab	7.76 ± 2.37 ABb
	Ca:Mg	6.48 ± 0.82 **a	3.47 ± 0.76 Ab	4.27 ± 1.37 Aab	4.54 ± 0.56 Bab
	K:P	5.94 ± 0.37 ***a	5.74 ± 0.46 ABa	3.81 ± 0.34 Bb	4.89 ± 0.47 Bab
	N:K	2.48 ± 0.17 ***a	1.52 ± 0.52 Ba	2.33 ± 0.59 Aa	1.7 ± 0.75 ABa
Grasses	C:N	20.8 ± 1.09 *ab	14.22 ± 2.02 Ab	15.39 ± 2.05 Ab	25.05 ± 5.21 Aa
	C:P	234.79 ± 12.74 ***a	121.52 ± 22.74 Bb	117.3 ± 22.2 Bb	148.6 ± 13.54 Bb
	N:P	11.54 ± 0.6 *a	7.15 ± 1.27 Bb	8.48 ± 1.7 Aab	7.3 ± 1.19 Bb
	Ca:Mg	3.77 ± 0.26 **a	3.49 ± 0.35 Aa	2.69 ± 0.7 Aa	3.84 ± 0.34 Ba
	K:P	8.05 ± 0.41 ***a	8.56 ± 1.33 Aa	6.59 ± 0.81 Aa	8.47 ± 0.66 Aa
	N:K	1.49 ± 0.14 ***a	0.99 ± 0.19 Ba	1.39 ± 0.43 Aa	0.85 ± 0.12 Ba

Capital letters denote significant differences among three plant growth forms while lowercases denote differences among four communities ($p < 0.05$). (Star denotes significant differences of shrubs and grasses in SH. * $p < 0.05$, ** $p < 0.01$ *** $P < 0.001$)

(Elser et al. 2000) (Table S7). N content (22.48 mg/g) was higher than the national level (20.62 mg/g, 20.24 mg/g) (Han et al. 2005) (Table S7). Leaf C:P and C:N ratios were indexes of plant ability to assimilate and store C, which represent the growth status of plants (Elser et al. 2000; Vitousek et al. 2010). In the PO, CWM of C:N of grasses (25) was higher than the global average (22.5) (Reich and Oleksyn 2004). However, CWM of C:P of grasses (148.6) in the PO was lower than in the SH. This indicates in the PO, grasses and shrubs have higher growth efficiency when N is sufficient and also suggests complex nutrient patterns in shrubs and grasses of different plantations in karst areas.

Major types of karst-forming rocks contain minerals, such as dolomite [$CaMg(CO_3)_2$], calcite and aragonite (Ford and Williams 2013). Therefore, most native plants have higher Ca and Mg concentrations. In our study, Ca concentration for shrubs was lower while Mg concentration was higher than those in another study in the karst area, southwest China (Liu et al. 2014). Although there was no significant difference among the four communities, CWM Ca/Mg ratios of trees differed among plantations and of shrubs between the native shrubs and plantations. In addition, we found lower CWM Ca and Mg concentrations in PY and AJ compared to in the SH and PO. This indicated species in two plantations (PY and AJ) have low Ca tolerance or potentially decreased alkaline extent in soils. Ca and Mg have been found to be correlated due to similar functions in plant metabolism and photosynthesis, and their ratio can be an indicator of plant tolerance to Ca (Garten 1976; Mengel et al. 1982; Wei et al. 2018). Our results indicated plantations reduce Ca uptake of

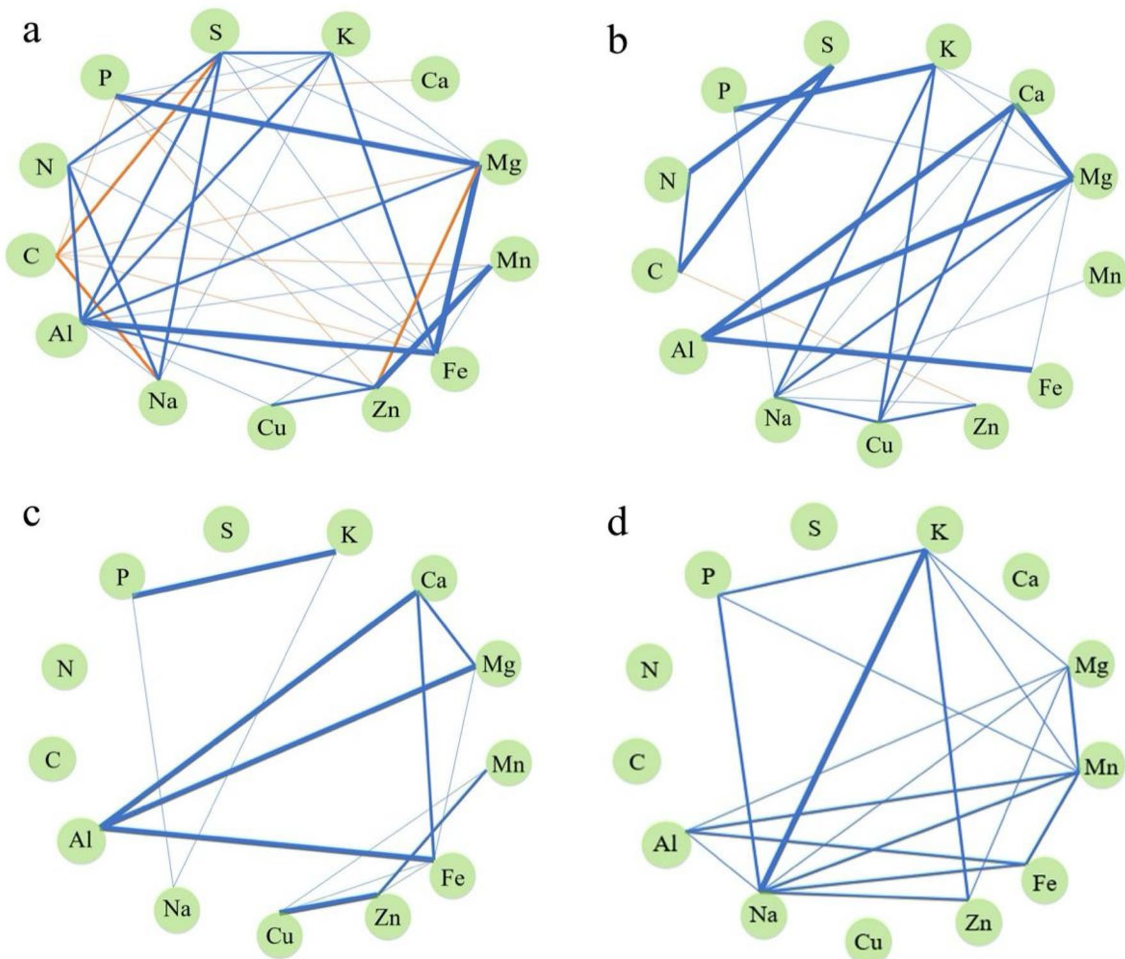

Fig. 5 Correlations between elements of plants in shrub community (**a**), Yunnan pine community (**b**), alder community (**c**) and cypress community (**d**). Blue line stands for the significant correlation between elements in plants and soils ($p<0.05$). Orange line stands for the negative relation, solid line stands for the positive relation. The thickness of the line represents the significance of the correlation ($p<0.05$, $p<0.01$, $p<0.001$)

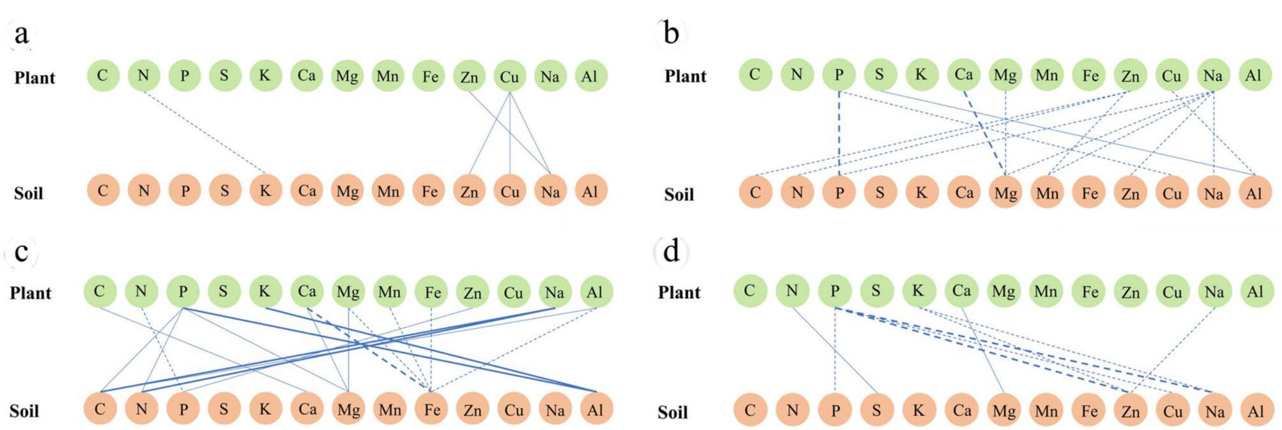

Fig. 6 Correlations between elements of plants and soils in the shrub community (**a**), Yunnan pine forest (**b**), alder forest (**c**) and cypress forest (**d**). Line stands for the significant correlation between elements in plants and soils ($p<0.05$). Dotted line stands for the negative relation, solid line stands for the positive relation. The thickness of the line represents the significance of the correlation ($p<0.05$, $p<0.01$, $p<0.001$)

shrubs which highlights the role of plantations in vegetation restoration and soil improvement.

K and Na are associated with cell water holding capacity (Mengel et al. 1982). A previous study found increased water input altered K stoichiometry of grasses in the karst area (Umair et al. 2020). However, few studies have investigated Na variation of plants in the karst area. Generally, K and Na are competitive to enter the plant (Mengel et al. 1982); however, our results showed K and Na were syngenetic. The specific mechanism was not clear at the moment. K concentration at native shrubs was the lowest indicating that plantations improved water holding capacity of plants. S concentration also varied between native shrubs and plantations in our study. Similar to N and P, S is a major element for the support of proteins and nucleic acids. Low S concentrations in the SH showed plantations improve plant growth and nutrient use efficiency.

Wright et al. (2004) supposed the concept of leaf economics spectrum (LES). LES illustrated a universal spectrum of leaf economics consisting of key chemical, structural and physiological properties. In our study, grasses had higher N, P concentrations and lower N:P while trees were in contrast. This is because quick-investment-return species (grasses) need more N and P to support quick growth and hence present higher N and P concentrations and lower N:P than trees. Although K is not often considered as a key factor in LES (Wright et al. 2005, 2006). Our result showed a clear K pattern: grasses > shrubs > trees in all communities. This addressed K role in dry karst area and hence, LES could be completed through adding local properties in regional studies.

In sum, we concluded N:P, Ca:Mg, K and S could be considered as key indicators to characterize plant nutrient status in native and shrubs and plantations at karst area, southwest China.

Change in limiting nutrient elements from the native shrub communities to plantation stands

Leaf N:P ratio, an index for N or P limiting of soils on plants, has been widely used to determine plant growth at individual, community and ecosystem levels (Elser et al. 2007). Various N:P ratios were used to determine N or P limiting based on vegetation and ecosystem types. For instance, Koerselman (1996) proposed an N:P ratio > 16 indicates P limitation at the community level, an N:P ratio < 14 indicates N limitation, and an N:P ratio between 14 and 16 indicates that either N or P can be limiting or that plant growth is co-limited by N and P together. A recent review by Güsewell (2004) provided a more conservative estimate of the N:P ratio threshold values: < 10 for N limitation and > 20 for P limitation. In our study, CWM of foliar N:P decreased from 11.92 for the native shrubs to 11.28, 10.15 and 9.72 in the three plantations, indicating that all three plantations establishment exacerbated N limitation and alleviate P limitation. Our results are similar to one previous report that plantations have the lowest N:P (6.57) compared to secondary forests (14.12) and natural forests in the karst area, southwest China (Song 2015). Natural forests are non-zonal climax community in the karst area with relatively balanced status between climate, vegetation and soils, which means that species in natural forests have higher nutrient use efficiency than plantations. Our results are different from a previous study that found plantations in southwest China are under P limitation (N:P 14 for *Eucalyptus maiden* and 19 for *Pinus yunnanensis*) (Pang et al. 2018). We tend to consider native shrubs are N-P co-limited, plantations are more N-limited, this is because disturbance of plantation would cause the loss of N and P, and N is usually lost more (Davidson et al. 2007). The regression analysis revealed N:P was associated with P in the native shrubs but was associated with N and P in the plantations (Fig. S1). This indicates that P cannot be ignored in southwestern China considering strong weathering and massive leaches of P in soils in the subtropical karst area (Townsend et al. 2007; Geekiyanage et al. 2019). These conflicting findings suggest that there is still large uncertainty due to different microclimates, heterogeneous soil, various vegetation characteristics, different nutrient use efficiencies, and management practices of plantations in the karst area. In addition, our study measured other typical elements for native shrubs and plantations, such as K. Therefore, we may consider using Olde Venterink's (Olde Venterink et al. 2003) nutrient limitation criterion (N:P > 14.5 plus K:P > 3.4 for N and P co-limitation, N:K > 2.1 plus K:P < 3.4 for N and K co-limitation, and N:P < 14.5 plus N:K < 2.1 for N limitation only) as a supplement to reduce uncertainty of nutrient limitation study in the karst area. In our study, K:P in four communities were all more than 3.4 but N:K were > 2.1 in all plantations. Hence, no K limitation was indicated in the current area.

In this study, grasses had the lowest N:P compared to trees and shrubs. A similar result was reported in another study in a karst area, Guangxi, China (Pan et al. 2015). When considering both growth form and communities, the determination of N or P limitation becomes more complex. Grasses in all four communities tended to be N-limited. Shrubs in the native shrubs were N-P co-limited while in the plantations were N-limited. Trees were N-P co-limited in the plantations. The regression analysis also revealed N:P ratios at growth form level were associated with plant N and P concentrations (Figs. S2, S3, and S4). Low N:P of plantations was mainly due to low N concentrations and N:P of shrubs and grasses. Higher N:P of trees in the plantations could reflect the dominance of trees in plantations under human disturbances, and hence trees took up more nutrients than shrubs and grasses at the community level. *A. japonica* can convert gaseous N_2 in the atmosphere into ammonia in the soil through N fixation for symbiosis (Seo et al. 2011). It is possible that *A. japonica* took more nitrogen and influenced community level N:P ratio.

We compared 12 foliar N:P studies in karst ecosystems across temperate, subtropical, and tropical areas (Fig. 7). The main factor controlling N:P at a large scale was latitude, consistent with the previous study across all ecosystems (Güsewell 2004). At the growth form level, the N:P order was trees > shrubs > grasses, which was consistent with 753 species investigation in China (Han et al. 2005). One recent study revealed nutrient limitation shifted from N to P from the early to the late stage of a short-term, secondary vegetation succession in a karst ecosystem (Zhang et al. 2015), similar to other ecosystems, such as tropical forests (Vitousek et al. 1993; Davidson et al. 2007), subtropical monsoon ecosystem (Huang et al. 2013), but not boreal ecosystem (Bormann and Sidle 1990; Uliassi and Ruess 2002). However, limited available data on N:P in the native forest/shrubs and plantations impaired our understanding of nutrient limitation under human disturbances and vegetation restoration.

Potential feedbacks to soils under the plantations

One important goal of plantations in karst areas is to improve soil quality, habitat environment, and finally foster vegetation recovery. One study showed vegetation restoration altered soil C, N and P concentrations based on specific species (Li et al. 2018). Another study found that the pine plantations have the lowest soil quality index compared to the bamboo plantations (intermediate soil quality) and a natural old growth forest (high soil quality), and hence concluded that pure plantation of *Pinus massoniana* has a negative impact on soil quality. These findings indicate that plant diversity and dominant tree species in plantations are important to improve vegetation restoration policy in the karst area. Our results revealed that plantation slightly modified the soil stoichiometry (Table S8, S9 and S10). Weak relationship between plant element in the native shrubs than in plantations was due to that native species tended to uptake nutrients directly from rocks with thin and poor soil during

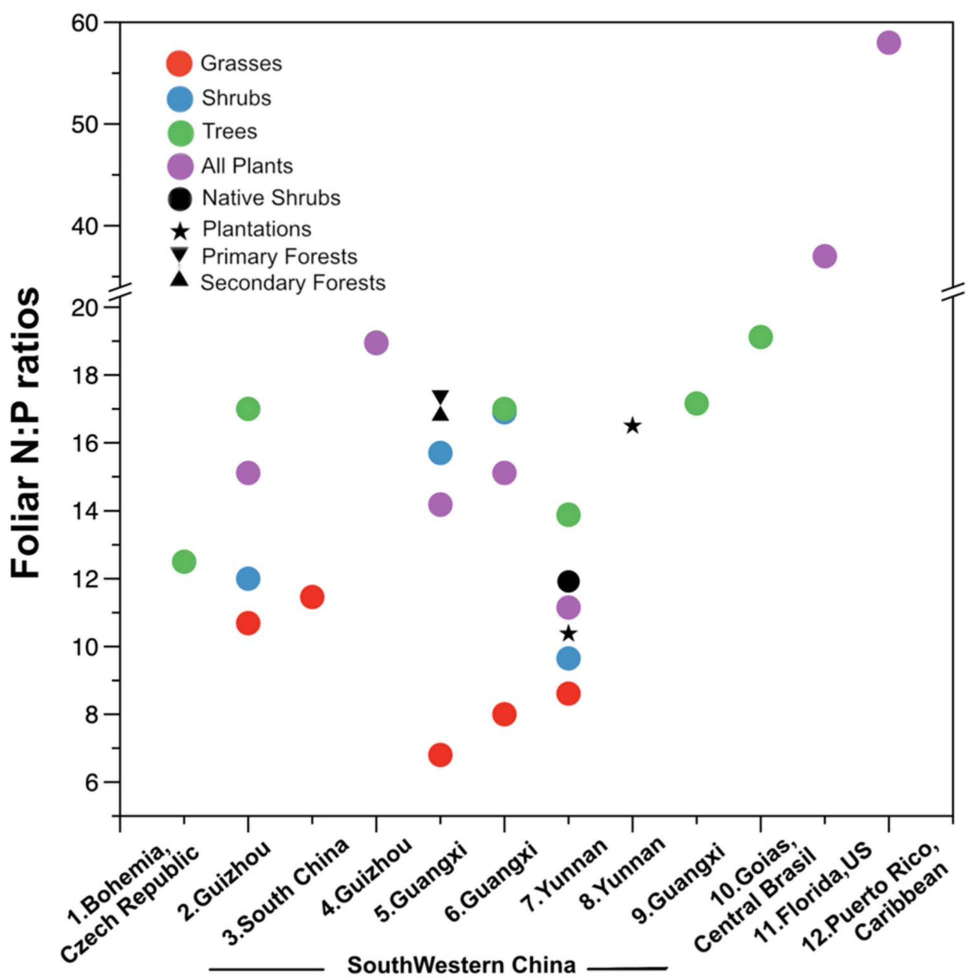

Fig. 7 Comparison of foliar N:P ratio in the karst ecosystem. From left to right on X-axis indicate publications from medium latitude (temperate karst area) to low latitude (tropical karst area). Different colors of the circle indicate data based on plant growth forms while different shapes indicate plantation stand type. Data were obtained from 1. Hofmeister et al. 2002, 2. Liu et al. 2014, 3. Kang et al. 2015, 4. Du et al. 2011, 5. Zhang et al. 2015, 6. Pan et al. 2015, 7. the present study, 8. Pang et al. 2018, 9. Bai et al. 2019, 10. Rossatto et al. 2015, 11. Saha et al. 2009, 12. Medina et al. 2017

long-time evolution. Stronger relationships between plant elements in the PY and AJ suggest that there are more complex species distribution in these two plantations with more steady community structure. We did not find significant difference of soil total N and P among the four communities but significant differences in ammonium concentrations were found among the SH, AJ and PO and significant differences in nitrate concentrations between the SH and PO. Soil ammonium, a major portion of mineral nitrogen in the shrubs and herb, could be directly absorbed and utilized by plants and usually not immobilized by microorganisms (Nordin et al. 2001). We think high soil ammonium in the AJ stand could result from the alder, which is a N-fixation species (Beaupied et al. 1990); however, current experiment design cannot confirm this, and more specific experiments need to be done in future. In addition, lower soil C, S, K and higher soil exchangeable Ca indicate that the PO had a negative influence on soil nutrients while the AJ seemed to be the best plantation among the three and had a positive influence on soil (particularly on avoiding N leaching).

Conclusion

By measuring multiple-element concentrations in leaves and soils in native shrub community and three plantations in the karst ecosystems Southwest China, we quantified differences in leaf elemental stoichiometry between plantation and native shrub community. Our results showed that (1) Native shrubs had lower leaf N, P, S, and higher C, Ca, and Mg concentrations, as well as C:P and N:P ratios, compared to plantations. (2) For different plant species, grasses had higher P, K, and Na and lower C:P and N:K, compared to trees and shrubs. (3) N:P in native shrubs was close to 12 while decreased to 11.3, 10.2 and 9.7 in three plantations. There is no K limitation in the current region (N:K < 2.1 and K:P > 3.4). The findings generated from this multiple-element stoichiometry study have some important implications. P limitations alleviate in plantations compared to native shrubs. Our study provides valuable information for karst ecosystem management. Further work should examine stoichiometry on different plantations based on species and community variation.

Author contributions statement Conceptualization: Chunjiang Liu and Jiahao Wen; Methodology: Chunjiang Liu and Jiahao Wen; Experiments: Huimin Tao, Ningxiao Sun, Jiahao Wen, Muhammad Umair; Data analysis: Jiahao Wen and Huimin Tao; Writing—review and editing: Jiahao Wen, Huimin Tao, Baoming Du, Dafeng Hui and Chunjiang Liu.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00468-021-02096-w.

Acknowledgements This work was funded by National Key R&D Program of China (2017YFC0505501).

Funding This work was funded by National Key R&D Program of China (2017YFC0505501).

Compliance with ethical standards

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

Data availability statement The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Standards We don't have experiments on animals or human subjects. All experiments follow the laws in the current country.

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