

# Effects of nitrogen, phosphorus and potassium addition on the productivity of a karst grassland: Plant functional group and community perspectives

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## ABSTRACT

Rocky desertification is currently the most serious ecological and environmental problem in karst region of southwestern China. Its negative consequences for both natural ecosystems and the human inhabitants of the karst region have created a need for effective ecosystem restoration strategies, but success in these efforts has been limited. We hypothesized that scarcity of mineral nutrients could be a crucial factor in vegetation recovery, but relatively little information is available about the limiting roles of various mineral elements. We investigated responses of aboveground biomass and nutrient concentrations of a degraded karst grassland to nitrogen (N), Phosphorus (P) and Potassium (K) additions over a period of three years. Nutrient additions significantly increased aboveground biomass and nutrient concentrations for both the plant community and individual plant functional groups. Total aboveground biomass was significantly increased by N (by 35.6%), P (by 35.3%) and K (by 11.7%) fertilization over three years of nutrient additions. The interaction effects of year  $\times$  N and year  $\times$  P on total biomass were significant. Additions of N and P increased the biomass of grasses by 39.2% and 15.0%, respectively, and additions of P increased the biomass of forbs by 69.3%. The biomass of shrubs was significantly increased by P (by 111.3%), K (by 45.3%) and N (by 38.5%), and there were strong interaction effects of N, P and K on shrub biomass. P and K additions significantly increased the relative biomass of shrubs but decreased that of grasses, especially under the NPK treatment. Our results suggest that the productivity of degraded grassland in the karst region of China is co-limited by N, P and K, with N and P being the primary limiting factors. Among functional groups, grasses are mainly limited by N and P, forbs by P, and shrubs by all 3 elements, with P being the most limiting factor overall. Mineral fertilization stimulates plant growth and may be a useful tool in efforts to restore woody vegetation in degraded grasslands, thus counteracting the process of rock desertification in the karst region of southwestern China.

## 1. Introduction

The subtropical karst region of southwestern China, occupying about 0.51 million km<sup>2</sup>, is one of the largest regions developed on carbonate bedrock in the world (Jiang et al., 2014). The region is characterized by extremely slow soil formation from the underlying limestone, very shallow and patchy soils with a low water retention capacity, and high porosity of the underlying limestone rock (Zhu, 1997; Liu, 2009). Typical undisturbed karst vegetation is a mixed evergreen and deciduous broad-leaved forest (Guo et al., 2011). In past

decades, many karst forests have experienced varying degrees of degradation caused by human disturbances, such as deforestation, agricultural expansion, livestock overgrazing and fire (Liu, 2009). Rocky desertification, referring to the processes that transform a karst area covered by vegetation and soil into a rocky landscape, is the most serious ecological and environmental problem in this region (Jiang et al., 2014). A large number of restoration projects have been carried out to counteract this trend. Many of these projects, involving planting of either indigenous or exotic species, have not been successful because of a lack of knowledge about the ecophysiological responses of the

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plants to the main stresses they face in the harsh karst habitats. For a long time, drought stress has been considered to be the most important factor limiting plant growth and vegetation restoration in the karst region (Zhu, 1997; Liu, 2009), and a number of studies have focused on ecophysiological adaptations of karst plants to drought stress (Wei et al., 2007; Liu et al., 2010, 2011). However, recent studies have suggested that shortage of mineral nutrients resulting from the limited total soil mass could be a crucial factor restricting plant growth and ecosystem productivity in this region (Zhang and Wang, 2009; Guo et al., 2011). Indeed, several researchers have reported that the mean depth of topsoil on the karst hills is only about 2–9 cm (Zhang and Wang, 2009; Liu et al., 2013).

The deficiency of plant-available phosphorus (P), resulting from lowered mobility of P bound to calcium phosphates, may be one of the most important factors limiting plant productivity in calcareous soils (Niinemets and Kull, 2005; Piao et al., 2005), and this was found to be the case for limestone grassland in the UK (Wilson et al., 1995). However, the overall productivity of calcareous grassland in northwest Switzerland was limited by nitrogen (N), and legume growth was limited by P (Niklaus et al., 1998; Stöcklin and Körner, 1999). The wooded meadows on calcareous soils in Estonia (Niinemets and Kull, 2003, 2005) and calcareous grasslands in the Peak District of the UK (Morecroft et al., 1994), northern Switzerland (Köhler et al., 2001) and Germany (Storm and Süß, 2008) were found to be co-limited by N and P.

To date, a few studies on plant nutrient stoichiometry have indicated that plant growth in the karst region of southwestern China is limited by P or co-limited by N and P (Du et al., 2011; Liu et al., 2014). The availability of soil N, P and potassium (K) were reported to be the most important nutrient factors determining woody species distribution in this region (Zhang et al., 2011; Du et al., 2015). However, previous studies based on the critical N:P ratios (Koerselman and Meuleman, 1996; Güsewell, 2004) have not drawn consistent conclusions. For example, according to Du et al. (2011), P mainly limits vegetation growth during secondary succession, whereas Zhang et al. (2015) found that there was a shift from N-limitation in grassland to P-limitation in secondary and primary forests during vegetation succession. Thus, there is no direct evidence indicating which nutrient indeed limits vegetation productivity and recovery in this karst region.

Although the criterion of Koerselman and Meuleman (1996) has been applied to various ecosystems, the critical N:P ratios may vary among species and ecosystems (Tessier and Raynal, 2003; Drenovsky and Richards, 2004; Güsewell, 2004; Reich and Oleksyn, 2004). Published N:P ratio thresholds for N limitation range from 6.7 to 16, while those for P limitation range from 12.5 to 26.3 (Tessier and Raynal, 2003). For example, the N:P ratios of a wooded meadow on calcareous soils in Estonia, which was N and P co-limited, ranged from 5.6 to 7.5 (Niinemets and Kull, 2005). Further fertilization experiments are

needed to evaluate the application of published criteria to the karst ecosystems of southwestern China and to elucidate how nutrients limit vegetation recovery in this region (Zhang and Wang, 2009; Du et al., 2011; Liu et al., 2014). This is crucial for understanding plant-soil interactions and successional mechanisms of native karst vegetation (Zhang et al., 2015) as well as providing fundamental knowledge for revegetation programs (Guo et al., 2011).

We hypothesized that scarcity of mineral nutrients could be a crucial factor limiting vegetation recovery of degraded karst ecosystems in southwestern China. In this study, we conducted a 3-year fertilization experiment to investigate the responses of a degraded karst grassland to N, P and K addition in order to determine their relative importance in limiting the productivity of grassland in this region. We also expected that different plant functional groups would respond differently to nutrient additions.

## 2. Materials and methods

### 2.1. Study site

This study was carried out at Puding Karst Ecosystem Research Station, Chinese Academy of Sciences, in Guizhou Province, China (26°22'25"N, 105°45'30"E; 1214 m asl). This region is a representative landscape for karst plateau in southwestern China (Wang et al., 2013). Long-term mean annual precipitation and temperature of this region are 1390 mm and 15.1 °C, respectively. The study was located on a karst hill with a slope of 25°. The original karst forest, mixed evergreen and deciduous broad-leaved forest, was destroyed in the late 1950s and gradually degraded to a grassland due to continual human disturbances. This site has been protected from livestock grazing since 1991, but secondary succession from grassland to forest is very slow. The mean height of vegetation is 1 m and coverage is 80%. This community usually has three layers. The dominant layer of bunchgrass is dominated by *Themeda japonica* (Willd.) Tanaka and *Heteropogon contortus* (Linn.) Beauv. and contains *Bothriochloa bladhii* (Retz.) S. T. Blake, *Carex lanceolata* Boott and *Capillipedium parviflorum* (R. Br.) Stapf. The lower herbaceous layer consists of many forbs, such as *Potentilla chinensis* Ser., *Lotus corniculatus* Linn., *Micromeria biflora* (Buch.-Ham. ex D. Don) Benth., *Rostellularia procumbens* (L.) Nees, *Gerbera anandria* (L.) Sch.-Bip., *Senecio scandens* Buch.-Ham. ex D. Don, *Aster ageratoides* Turcz., *Viola verecunda* A. Gray and *Polygala tatarinowii* Regel. The scattered shrub layer contains *Rubus parvifolius* L., *Indigofera pseudotinctoria* Matsum., *Campylotropis macrocarpa* (Bge.) Rehd., *Lespedeza bicolor* Turcz., *Rosa cymosa* Tratt. and *Elsholtzia rugulosa* Hemsl.. The soil type is limestone soil, according to the Chinese soil genetic classification, similar to Rendolls in the USDA Soil Taxonomy (Soil Survey Staff, 1999). Soil pH is 7.7 and organic carbon content is 53.5 g kg<sup>-1</sup>. Soil nutrient properties are described in Table 1. Due to the

Table 1

Community and soil properties of karst grassland from four replicates of the control plots over three years (Means ± SE; n = 12). Different lowercase letters indicate significant differences among plant functional groups.

Community property	Grasses	Forbs	Shrubs	Community
Aboveground biomass (g m <sup>-2</sup> )	197.49 ± 9.24 a	36.65 ± 5.98 b	33.20 ± 5.50 b	267.34 ± 12.76
Proportion of biomass (%)	74.25 ± 1.89 a	13.78 ± 2.19 b	11.98 ± 1.71 b	
N (g kg <sup>-1</sup> )	11.24 ± 0.29 a	14.05 ± 0.88 b	13.51 ± 0.93 ab	11.88 ± 0.21
P (g kg <sup>-1</sup> )	0.88 ± 0.05 a	0.94 ± 0.06 a	0.93 ± 0.08 a	0.89 ± 0.04
K (g kg <sup>-1</sup> )	7.37 ± 0.39 a	8.74 ± 0.53 a	7.48 ± 0.57 a	7.49 ± 0.32
N:P	13.17 ± 0.74 a	15.08 ± 1.06 a	14.98 ± 0.91 a	13.67 ± 0.56
N:K	1.58 ± 0.10 a	1.64 ± 0.14 a	1.93 ± 0.19 a	1.64 ± 0.08
K:P	8.61 ± 0.57 a	9.63 ± 0.80 a	8.39 ± 0.70 a	8.67 ± 0.46
Soil property	Soil property			
Total N (g kg <sup>-1</sup> )	3.99 ± 0.21	Available N (mg kg <sup>-1</sup> )		373.33 ± 18.79
Total P (g kg <sup>-1</sup> )	0.90 ± 0.04	Available P (mg kg <sup>-1</sup> )		6.32 ± 0.51
Total K (g kg <sup>-1</sup> )	1.85 ± 0.13	Available K (mg kg <sup>-1</sup> )		86.41 ± 5.77

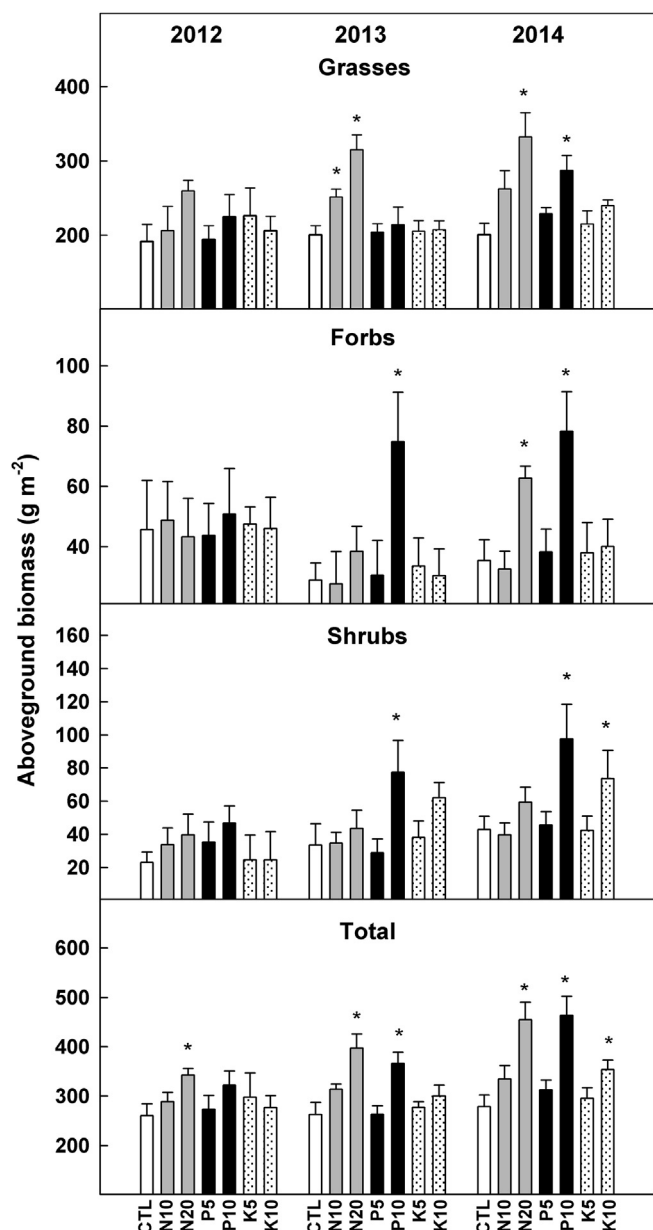


Fig. 1. Aboveground biomass responses to low and high additions of nitrogen (N), phosphorus (P), and potassium (K) over time. Bars are Mean  $\pm$  SE ( $n = 4$ ). Asterisks indicate significant differences between the treatments and control plots (CTL) in each year. Nutrient additions are 10 and 20 g N m $^{-2}$  for N10 and N20, 5 and 10 g P m $^{-2}$  for P5 and P10, and 5 and 10 g K m $^{-2}$  for K5 and K10, respectively.

patchy and shallow soil layer over bedrock (0–10 cm), the total nutrient stocks are very limited in this ecosystem.

## 2.2. Experimental design and fertilization treatments

We began a 3-year fertilization experiment in 2012, using a randomized block design with 4 blocks and 11 treatments: (1) Control, (2) N10, (3) N20, (4) P5, (5) P10, (6) K5, (7) K10, (8) N20 + P10, (9) N20 + K10, (10) P10 + K10, and (11) N20 + P10 + K10. N was added as urea ((NH $_2$ ) $_2$ CO), P as superphosphate (Ca(H $_2$ PO $_4$ ) $_2$ ·H $_2$ O), and K as potassium chloride (KCl). Blocks were 18  $\times$  24.5 m in size, separated by 20–30 m on the karst hill. The 11 treatment plots in each block were each 5  $\times$  5 m and separated from each other by a 1.5 m buffer. Annual doses were 10 g and 20 g N m $^{-2}$  yr $^{-1}$  for N10 and N20, respectively, 5 g

and 10 g P m $^{-2}$  yr $^{-1}$  for P5 and P10, respectively, and 5 g and 10 g K m $^{-2}$  yr $^{-1}$  for K5 and K10, respectively. Nutrients were added by hand in an even distribution in three events during each growing season.

## 2.3. Sampling and chemical analyses

Aboveground biomass was harvested annually in the middle of September from each experimental plot by hand-clipping vegetation at the soil surface in 1  $\times$  1 m quadrats, with no spatial overlap of the successively harvested areas. All clipped plants were sorted into three plant functional groups: grasses, forbs and shrubs (including small/young trees). All samples were oven-dried at 65  $^{\circ}$ C to constant weight, weighed and stored for further chemical analyses. Topsoil samples (0–10 cm) were collected at the time of biomass sampling. Soil samples were air-dried in the field before being shipped to the laboratory for analysis. All the samples were analyzed following the procedures described by Dong et al. (1996) and Liu (1996). Total N concentration was determined using the Kjeldahl method, and Total P and K concentrations were determined with an inductive coupled plasma (ICP) emission spectrometer after digestion of the samples in concentrated HNO $_3$ . Soil alkali-hydrolysable N (available N, or AN) was determined by titration with a dilute solution of H $_2$ SO $_4$  after extraction with a mixture of ferrous sulfate and sodium hydroxide. Available phosphorus (AP) was determined by molybdate blue colorimetry after extraction with sodium bicarbonate. Available K (AK) was determined with ICP after extraction with ammonium acetate.

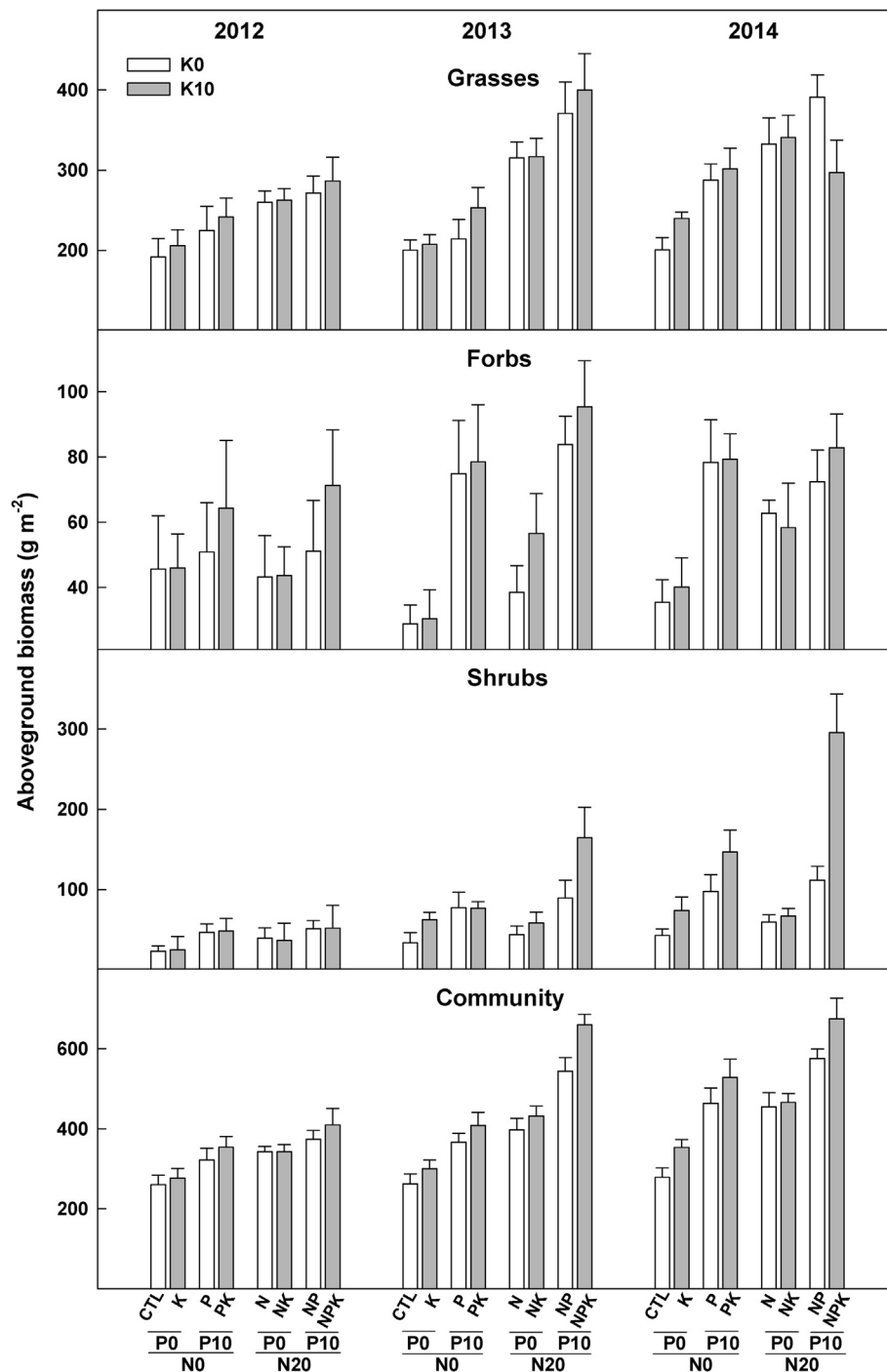
## 2.4. Data analyses

We calculated community-level nutrient concentrations and ratios of aboveground biomass as weighted averages calculated from the mean values for different functional groups and their proportional contributions to total biomass. Because N, P and K levels were not fully factorial in our experimental design, we analyzed data in two main steps. First, we tested the responses of the grassland to different supply levels of each nutrient. Data from plots with single nutrient (N or P or K) additions were subjected to repeated measures ANOVA to determine differences among nutrient supply levels across the three years, with year as a within-subject effect and supply level (control, low and high levels) as a between-subject effect. Second, we used the data from plots with separate and combined high nutrient additions (i.e., control, N20, P10, K10, N20 + P10, N20 + K10, K10 + P10 and N20 + K10 + P10) to make up a fully factorial dataset and test N  $\times$  P  $\times$  K interaction effect. We also performed repeated measures ANOVA for this dataset, with year as a repeated effect and N, P and K treatments as the main effects. Between-subject effects determined the responses to the additions of the three nutrients over time, while within-subject effects evaluated variation among years and interactions among treatments and year. The Greenhouse-Geisser adjusted F-statistic was used to test the significance of all within-effects. The relationships between aboveground biomass and nutrient concentrations and soil available nutrient concentrations were determined by Pearson's correlation and linear regression. Data on biomass and nutrients were log-transformed to satisfy the assumption of normality. All effects were considered significant at  $p < 0.05$ . Statistical analyses were performed using the R statistical platform (R Development Core Team, 2012) and SPSS version 17.0 (SPSS, USA).

## 3. Results

### 3.1. Soil nutrients

Soil nutrient properties in control plots are presented in Table 1. Total and available N, P and K concentrations were significantly increased by adding N, P and K, respectively (Figs. A.1–2; Table A.1).



**Fig. 2.** Aboveground biomass responses to separate and combined high additions of nitrogen (N), phosphorus (P), and potassium (K) over time. Bars are Mean  $\pm$  SE ( $n = 4$ ). CTL represents control treatment. Nutrient additions are 0 and 20 g N m<sup>-2</sup> for N0 and N20, 0 and 10 g P m<sup>-2</sup> for P0 and P10, and 0 and 10 g K m<sup>-2</sup> for K0 and K10, respectively.

Usually, low nutrient additions did not significantly increase soil nutrient concentrations, except the significant effect of low P supply on soil available P (Fig. A.1). Available nutrients appeared to be more sensitive to fertilization treatments than total nutrients. The interactions of year  $\times$  N  $\times$  P  $\times$  K on total P, of year  $\times$  P on AP and of year  $\times$  K on AK were also significant (Table A.1; Fig. A.2).

### 3.2. Aboveground biomass

Total aboveground biomass was significantly increased by the high additions of N, P and K and increased over time (Fig. 1). Weak between-

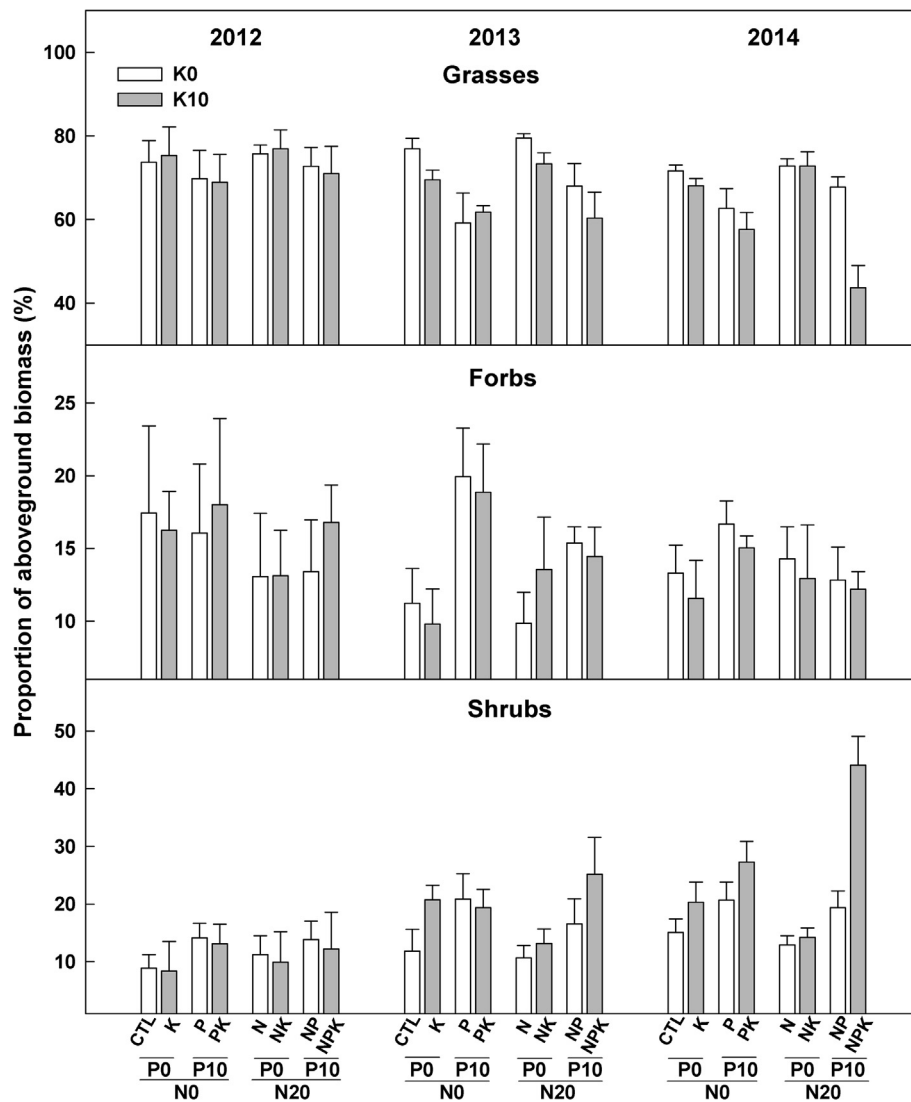
subject interaction effects of P  $\times$  K and N  $\times$  P  $\times$  K were observed for total biomass ( $p < 0.1$ ; Fig. 2; Table 2), as were significant within-subject interaction effects of year  $\times$  N and of year  $\times$  P ( $p < 0.01$ ; Table 2). At the end of the experiment, total biomass was highest under the NPK treatment, 1.42 times greater than that of the control (Fig. 2).

At the plant functional group level, the aboveground biomass of grasses and forbs were significantly increased by high additions of N and P after three years; biomass of shrub was significantly increased by high additions of P and K (Fig. 1). The three-way interaction of N, P and K was significant for shrubs (Fig. 2; Table 2). Other significant interaction effects were for year  $\times$  N for grasses, and for year  $\times$  P, year  $\times$  K

**Table 2**

Effects of N (0 and 20 g m<sup>-2</sup>), P (0 and 10 g m<sup>-2</sup>) and K (0 and 10 g m<sup>-2</sup>) addition over time (year) on aboveground biomass based on repeated measures of analysis of variance. Shown are the F-values with the significance levels: <sup>a</sup>P < 0.1, \*P < 0.05, \*\*P < 0.01, and \*\*\*P < 0.001.

Effect	d.f.	Aboveground biomass				Proportions of biomass		
		Total	Grasses	Forbs	Shrubs	Grasses	Forbs	Shrubs
N	1,24	180.87***	99.088***	3.477 <sup>a</sup>	15.162**	0.882	1.85	0.021
P	1,24	184.238***	18.53***	37.755***	73.532***	36.476***	4.51*	26.036***
K	1,24	25.616***	0.737***	1.972	23.307***	6.409*	0	8.783**
N × P	1,24	0.681	0.663	0.637	7.811*	0.329	1.197	2.765
N × K	1,24	0.076	2.44	0.306	4.303*	1.54	0.299	0.9
P × K	1,24	3.756 <sup>a</sup>	0.241	0.474	8.051**	1.176	0.022	1.272
N × P × K	1,24	3.089 <sup>a</sup>	0.443	0.082	9.666**	2.965 <sup>a</sup>	0.05	4.881*
Year	2,48	42.562***	9.09**	1.798	28.544***	6.436**	0.831	14.521***
Year × N	2,48	6.778**	4.184*	0.693	1.604	0.46	0.188	0.464
Year × P	2,48	7.565**	0.379	2.689 <sup>a</sup>	9.898**	2.093	1.56	2.547 <sup>a</sup>
Year × K	2,48	1.111	0.55	0.131	6.419**	1.631	0.248	3.805*
Year × N × P	2,48	1.773	1.921	0.698	2.705a	0.463	0.84	1.775
Year × N × K	2,48	0.383	0.88	0.118	1.185	0.277	0.064	0.541
Year × P × K	2,48	0.042	1.611	0.267	3.714*	1.657	0.361	2.164
Year × N × P × K	2,48	0.219	0.369	0.139	2.059	0.752	0.139	1.085



**Fig. 3.** Proportions of aboveground biomass of the three functional groups of the total community biomass under separate and combined high additions of nitrogen (N), phosphorus (P), and potassium (K). Bars are Mean  $\pm$  SE (n = 4). Figure annotations are the same as in Fig. 2.



**Table 3**

Effects of N (0 and 20 g m<sup>-2</sup>), P (0 and 10 g m<sup>-2</sup>) and K (0 and 10 g m<sup>-2</sup>) addition over time (year) on nutrients in aboveground biomass of total community based on repeated measures of analysis of variance. Shown are the F-values with the significance levels: \*P < 0.1, \*\*P < 0.05, \*\*\*P < 0.01, and \*\*\*\*P < 0.001.

Effect	d.f.	N	P	K	N:P	N:K	K:P
N	1,24	204.684***	0.446	1.448	85.474***	72.414***	0.111
P	1,24	2.326	444.843***	0.06	472.996***	2.28	205.266***
K	1,24	8.932**	4.043 <sup>a</sup>	285.757***	7.158*	143.587***	141.463***
N × P	1,24	0.16	0.058	3.372	9.834**	3.885 <sup>a</sup>	0.583
N × K	1,24	0.153	1.203	0.294	0.134	7.57*	0.156
P × K	1,24	2.728	3.045 <sup>a</sup>	3.85 <sup>a</sup>	4.19 <sup>a</sup>	1.259	3.24 <sup>a</sup>
N × P × K	1,24	0.39	1.785	0.279	2.593	1.268	0.73
Year	2,48	26.866***	49.621***	21.395***	14.608***	0.837	10.135**
Year × N	2,48	9.268**	2.921 <sup>a</sup>	0.058	1.582	3.76*	0.572
Year × P	2,48	4.302*	55.013***	1.356	25.958***	0.316	18.474***
Year × K	2,48	2.109	0.636	30.001***	0.921	11.201***	8.457**
Year × N × P	2,48	0.222	2.078	0.149	1.41	0.009	0.281
Year × N × K	2,48	0.112	0.44	1.166	0.09	3.57*	0.777
Year × P × K	2,48	2.586	0.007	2.238	0.302	0.299	2.381
Year × N × P × K	2,48	0.076	0.071	0.174	0.082	0.124	0.389

and year × P × K for shrubs (Table 2). The highest biomasses of grasses and forbs were observed under the NPK treatment in 2013, increasing by 1.00 and 2.31 times, respectively, whereas the highest shrub biomass was observed in 2014, increasing by 5.89 times (Fig. 2).

The relative biomasses of the three functional groups were mainly affected by P and K additions, as indicated by their proportions of biomass to the total community biomass (Fig. 3; Table 2). P and K additions significantly increased the relative biomass of shrubs but decreased that of grasses, while P addition also increased the relative biomass of forbs. There was a significant N × P × K interaction on the relative biomass of shrubs (Table 2). The biomasses of both grasses and shrubs significantly increased over time (Figs. 1 and 2; Table 2). However, the relative biomass of grasses decreased significantly with time and that of shrubs increased, especially under fertilization treatments (Fig. 3; Table 2).

### 3.3. Plant nutrients

Mean N, P and K concentrations for total biomass in control plots across three years were 11.88, 0.89 and 7.49 g kg<sup>-1</sup> and the N:P, N:K and K:P ratios were 13.67, 1.67 and 8.67, respectively. There were no significant differences in concentrations of the three elements among the three functional types, with the exception that forbs had higher N concentrations than grasses (Table 1). There were also no significant differences in nutrient ratios among the functional groups (Table 1). Both low and high additions of N, P and K significantly increased biomass N, P and K concentrations, respectively, and affected the related nutrient ratios (Fig. A.3). The N:P and N:K ratios were affected by N × P and N × K interactions, respectively (Table 3; Fig. 4). There were significant variations in the biomass nutrient concentrations and ratios among years, except for the N:K ratio (Table 3). Moreover, there were significant interactions of year × N on N concentration and N:K ratio, of year × P on N and P concentrations and N:P and K:P ratios, of year × K on K concentration and N:K and K:P ratios, and of year × N × K on N:K ratio (Table 3).

The three functional groups showed responses similar to that of total community biomass to nutrient additions. N, P and K concentrations in three functional groups were increased by the addition of N, P and K (Fig. 5; Repeated-measures ANOVA: between-subject effects of N, P and K,  $p < 0.001$ ). There were significant and positive interaction effects of year × N on N concentration, of year × P on P concentration, and year × K on K concentration in all the plants (within-subject effects of year × N (or P or K):  $p < 0.01$ ), and the year × N interaction also increased P concentration in forbs (within-subject effect of year × N on P:  $F_{2, 48} = 4.149$ ,  $p < 0.05$ ). After three years of fertilization, average N concentration under treatments with N addition increased by 41.9%

in grasses, 35.5% in forbs and 75.8% in shrubs, respectively. P addition increased P concentrations in the three functional groups by 161.9–186.9% and K addition increased K concentrations by 91.6–104.2% (Fig. 5).

### 3.4. The relationship between soil nutrient availability and plant biomass and nutrients

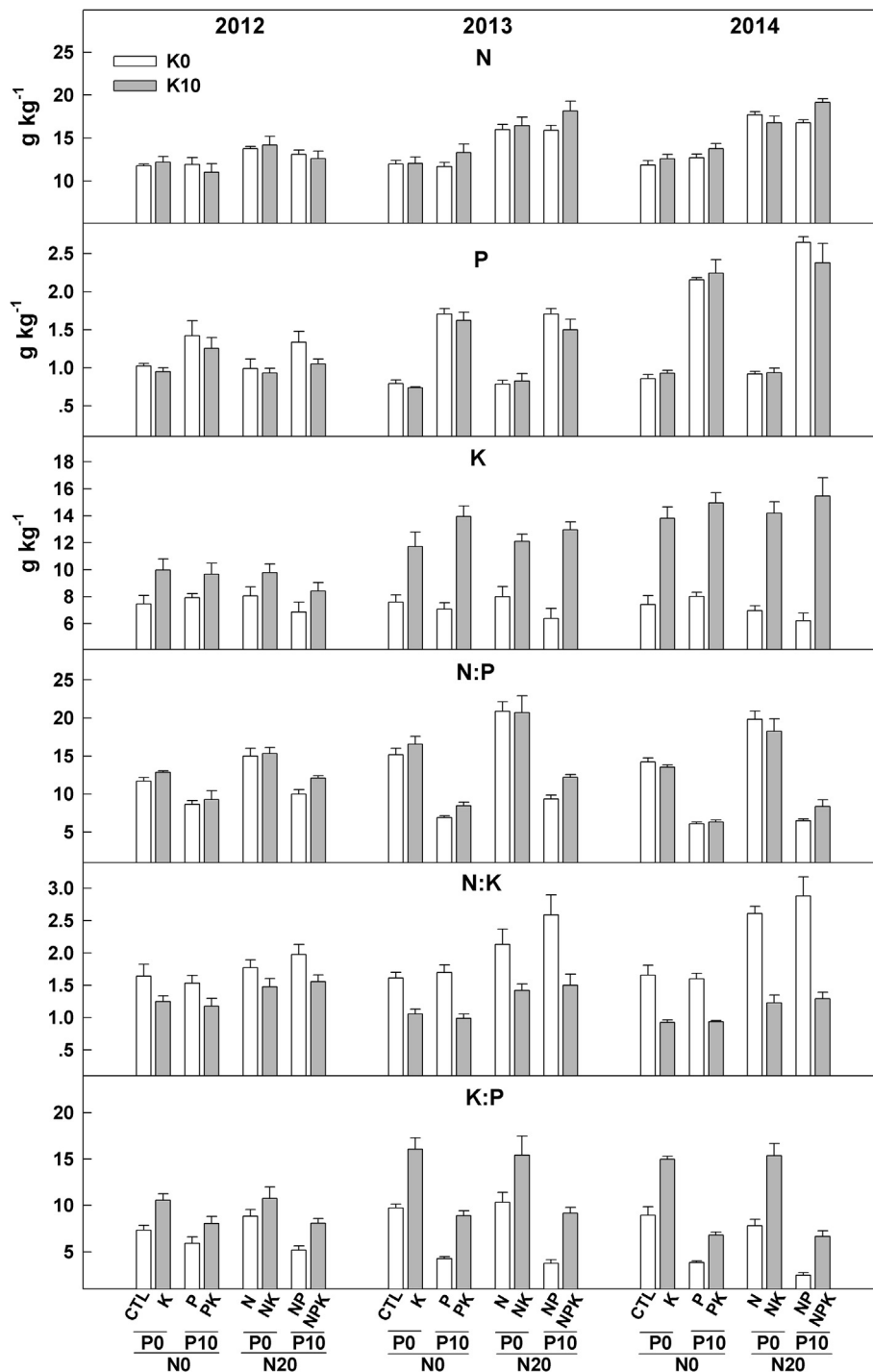
There were significant and positive relationships between above-ground biomasses of the plant community and all functional groups with soil available N and P, and between biomasses of the plant community and shrubs with soil available K (Fig. 6). The proportion of grass biomass was negatively correlated with soil available P and K, while the proportion of shrub biomass was positively correlated with soil available N, P and K. The relative biomass of forbs was negatively correlated with soil available N and was positively correlated with soil available P.

Concentrations of N, P and K in the three functional groups increased with soil available N, P and K, respectively (Fig. 7). N:P and N:K in all plants decreased with soil available P and K, respectively. K:P in all plants decreased with soil available P and increased with soil available K. N:K in grasses increased with soil available N.

## 4. Discussion

### 4.1. Nutrient limitations to the karst grassland community

Our study demonstrated that separate additions of N, P or K significantly increased total aboveground biomass of the karst grassland (Figs. 1 and 2; Table 2) and that there were weak interaction effects of P × K and N × P × K on the total biomass. Moreover, the aboveground biomass significantly increased with increasing amounts of soil available N, P and K (Fig. 6), suggesting a co-limitation by the three nutrients to the productivity of the karst grassland in this region. Across all treatment combinations over three years, the mean effect on total aboveground biomass was +35.6% for N, +35.3% for P and +11.7% for K, indicating that N and P were the primary limiting factors and K a secondary factor. Many previous studies have reported co-limitation of grassland productivity on calcareous soils by N and P (Morecroft et al., 1994; Köhler et al., 2001; Storm and Süß, 2008); Niinemets and Kull (2003, 2005) reported similar effects for wooded meadows on calcareous soils. Our study provides evidence that, in addition to N and P, K could be another limiting nutrient on calcareous substrates. K limitation has also been found in peatlands (Wang et al., 2016), mires (Olde Venterink et al., 2009), wet meadows (Olde Venterink et al., 2001; Van de Riet et al., 2010), grasslands (Fay et al., 2015) and tropical forests (Wright et al., 2011; Santiago et al., 2012). K limitations however have



**Fig. 4.** Nutrient concentrations and ratios in aboveground biomass of the total community under separate and combined high additions of nitrogen (N), phosphorus (P), and potassium (K). Bars are Mean  $\pm$  SE ( $n = 4$ ). Figure annotations are the same as in Fig. 2.

been largely ignored and should receive more attention (Wright et al., 2011; Lannes et al., 2016).

Nutrient limitations can also be diagnosed by study of plant nutrient concentrations and ratios (Van de Riet et al., 2010). In the present study, mean N, P and K concentrations in aboveground biomass in control plots were lower than the physiological concentration requirements of N ( $15 \text{ g kg}^{-1}$ ), P ( $2 \text{ g kg}^{-1}$ ) and K ( $10 \text{ g kg}^{-1}$ ) for adequate plant growth (Marschner, 1995), suggesting that the productivity of this karst grassland would potentially be co-limited by the three elements. Koerselman and Meuleman (1996) have proposed that a vegetation N:P ratio  $< 14$  indicates N limitation, while a ratio  $> 16$  suggests P

limitation. At N:P ratios between 14 and 16, either N or P can be limiting or plant growth may be co-limited by N and P. The mean biomass N:P ratio under the control treatment was 13.67 in this study, indicating an N limitation according to these criteria. However, our results showed that both N and P fertilization significantly increased biomass over the 3-year course of the study. Güsewell (2004) has suggested more conservative N:P thresholds,  $< 10$  for N limitation and  $> 20$  for P limitation, based on an extensive review of fertilization studies across different vegetation types. The N:P ratio of 13.67 for our control plots falls in the range of 10–20, indicating that it would be safer to use the N:P thresholds of Güsewell in this karst habitat. Du et al.

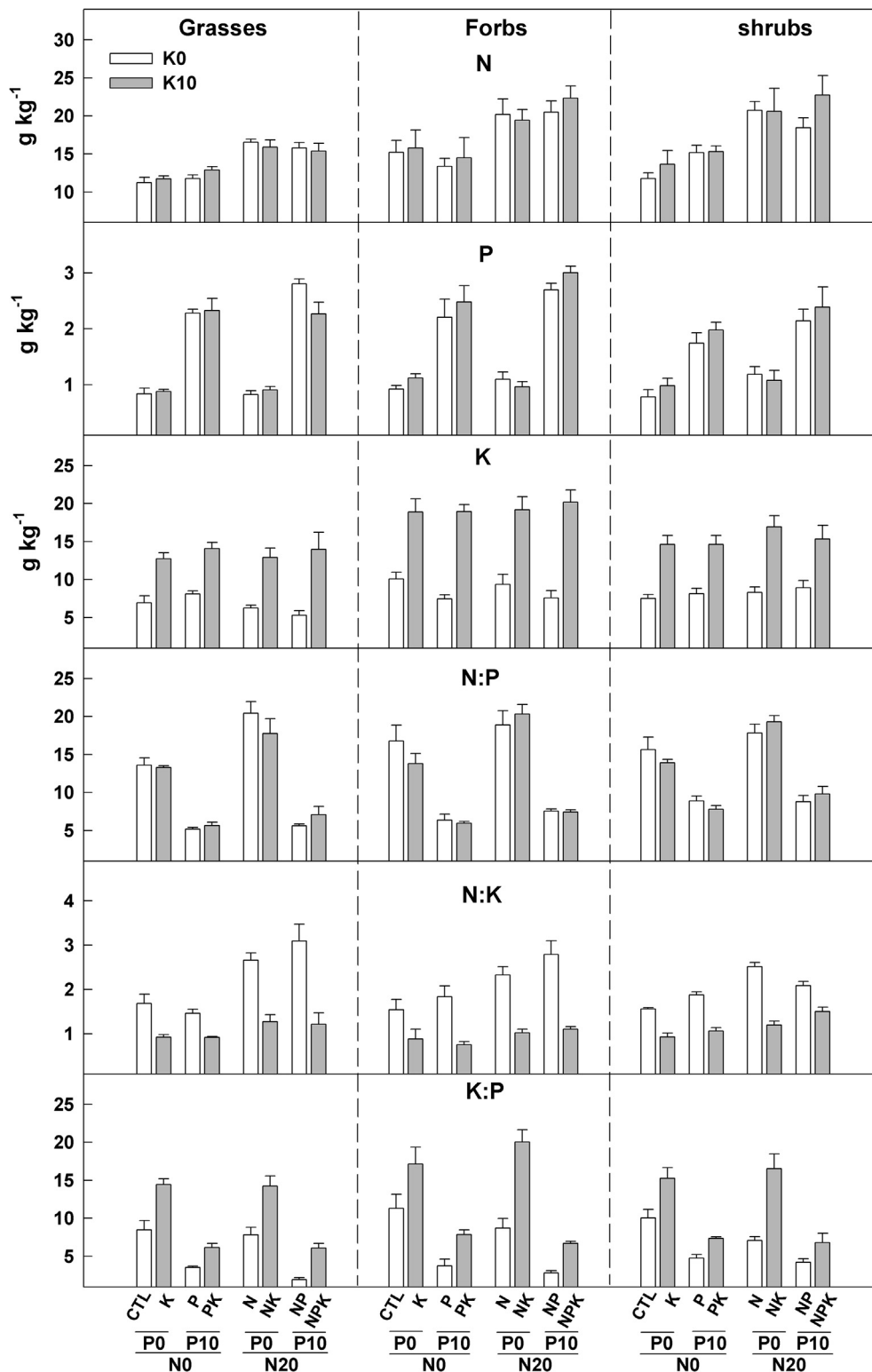
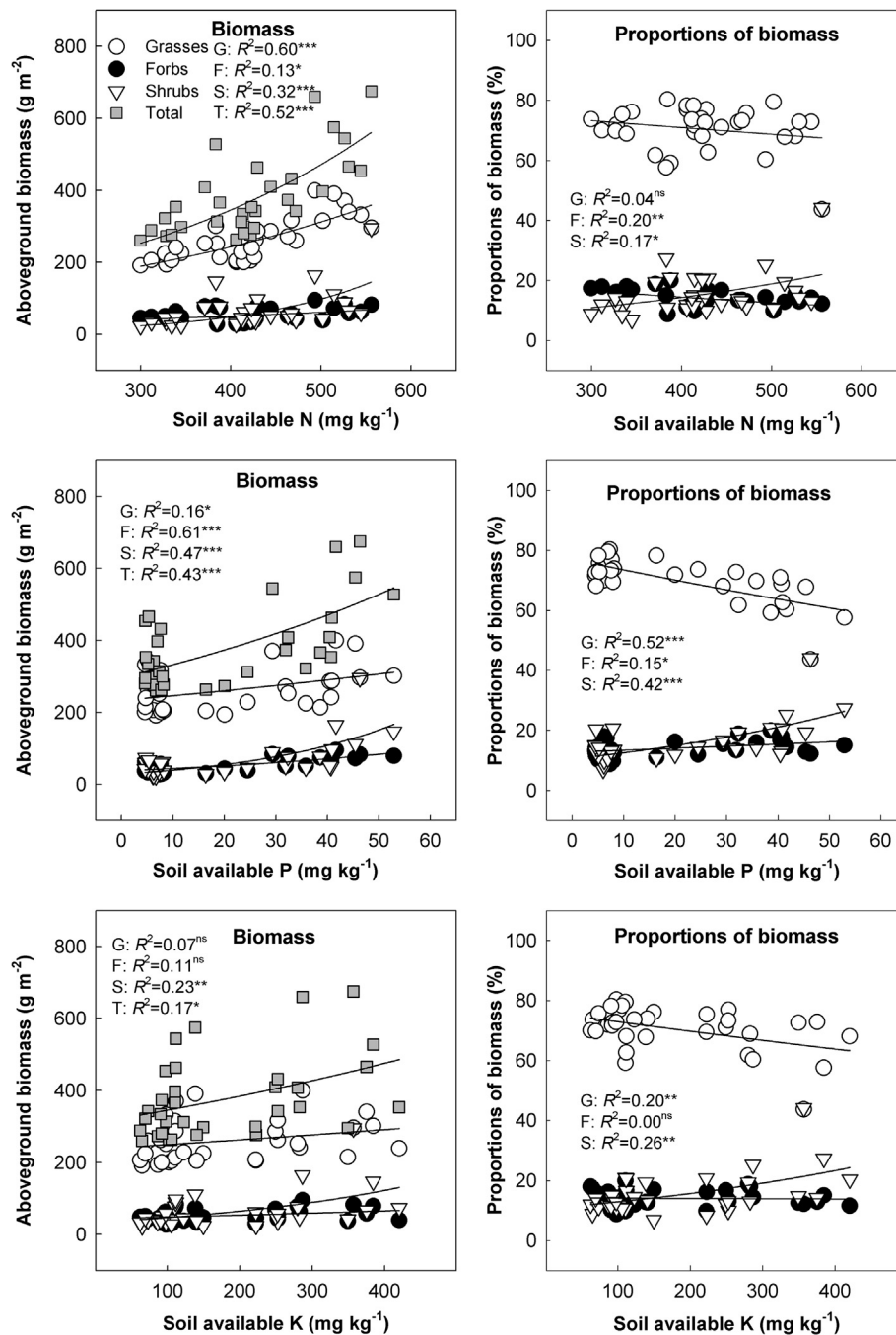


Fig. 5. Nutrient concentrations and ratios in different plant functional groups after three years (2014) of high additions of nitrogen (N), phosphorus (P), and potassium (K). Bars are Mean  $\pm$  SE ( $n = 4$ ). Figure annotations are the same as in Fig. 2.

(2011) also suggested that N limitation would be predicted at N:P ratios below 10 and P limitation at N:P ratios over 18 in this region. According to Olde Venterink et al. (2003), plant N:K > 2.1 and K:P < 3.4 indicate K limitation. In our study, the mean biomass N:K and K:P ratios for the control plots were 1.64 and 8.67, respectively, suggesting that K would not be a limiting factor. But the ratios of N:K (2.88) and K:P (2.46) under the N + P treatment in 2014 did meet the criterion of K

limitation. Our experiment also detected significant increases of biomass and K concentration caused by K addition. As with the critical N:P ratios (Tessier and Raynal, 2003), the threshold values of N:K and K:P ratios for K limitation may also vary among species and ecosystems, which has not been extensively tested. Moreover, plant N:P and N:K ratios depend more on soil available P and K than on soil available N (Fig. 7), indicating that the relative limitations were more controlled by





**Fig. 6.** The relationship between soil available N, P and K concentrations and aboveground biomass and proportions of biomass of the three functional groups. Values are Mean  $\pm$  SE (n = 4). Abbreviations are: Grasses (G), Forbs (F), Shrubs (S), and total community (T). Significant level: \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, <sup>ns</sup>not significant.

P and K than N. One possible explanation is that this area receives a high atmospheric N deposition ( $3.1 \text{ g m}^{-2} \text{ yr}^{-1}$ ) (Liu et al., 2008), while P and K mostly come from rock weathering. However, P is typically bound in calcium phosphates in calcareous soils (Ninemets and Kull, 2005; Piao et al., 2005), and K is particularly prone to leaching losses in humid areas (Wright et al., 2011).

The significant and positive relationships between soil available nutrients and vegetation biomass and nutrient concentrations indicated that the productivity of the karst grassland at our study site, to some extent, depends on soil nutrient status. Limitation of vegetation growth by multiple nutrients (N, P and K) is likely due to the shallow soil layer. The grasslands in the karst regions of southwestern China usually develop on abandoned farmlands or after the destruction of forests (Guo

et al., 2011; Zhang et al., 2015). Stocks of soil nutrients have been substantially reduced in the process of biomass removal and soil erosion following deforestation. For example, soil total N and P in degraded grasslands decreased from their levels in primary forests by 72% and 54% (Zhang et al., 2015). Because there are few acid insoluble materials in carbonate bedrock, the soil formation rate is extremely slow (Wei, 1996) and the inputs of many nutrients are extremely limited (Zhang and Wang, 2009). Moreover, this region receives about 1400 mm annual precipitation, most of which is concentrated in the growing season. Nutrient leaching is a serious problem because of the high porosity of the underlying carbonate rock, especially for highly mobile elements like K.

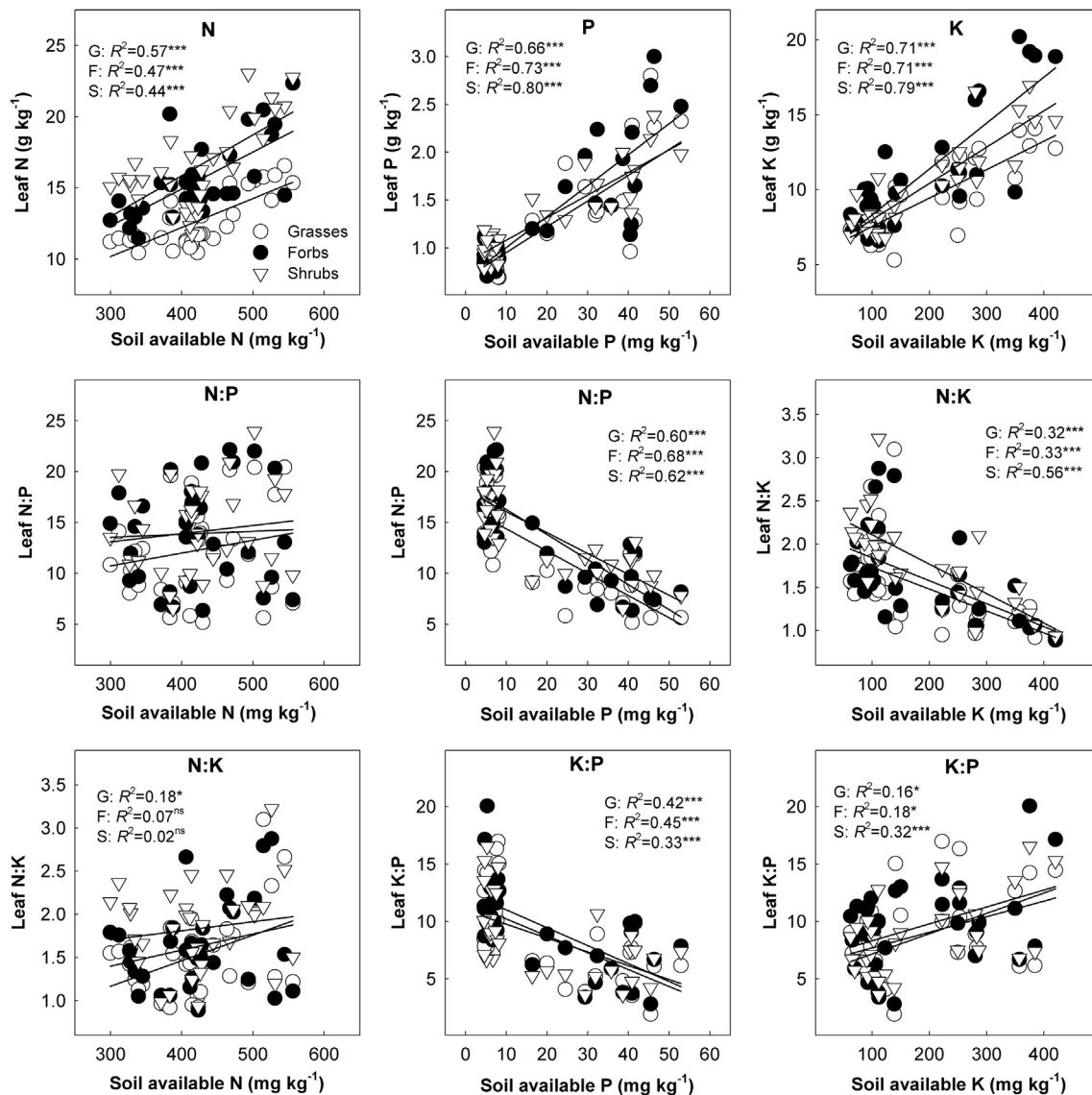


Fig. 7. The relationships between soil available N, P and K concentrations and nutrient concentrations and ratios in the three functional groups. Values are Mean  $\pm$  SE ( $n = 4$ ). Abbreviations are: Grasses (G), Forbs (F), Shrubs (S). Significant level: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , <sup>ns</sup>not significant.

#### 4.2. Different plant functional groups were mainly limited by different nutrients

Although the three plant functional groups had similar stoichiometric properties (Table 1), forb biomass was only significantly increased by P addition (by 69.3%) across all treatment combinations over three years, while shrub biomass was significantly increased by P (by 111.3%), K (by 45.3%) and N (by 38.5%) additions, and there were strong interaction effects of the three elements on shrubs. The biomass of forbs increased with soil available P and N, while biomass of shrubs increased with soil available P, N and K. This indicated that forbs were mainly limited by P and shrubs were co-limited by the three nutrients in the order of  $P > K$  and N. Biomass of grasses increased with N (by 39.2%) and P (by 15.0%) additions, and it also increased with soil available N and P, indicating that grasses were mainly limited by N, followed by P. Such differential limitation by different nutrients within a plant community has also been found in many other ecosystems, such as European calcareous grasslands (Willems et al., 1993; Niklaus et al., 1998; Stöcklin and Körner, 1999; Köhler et al., 2001; Storm and Süss, 2008), arctic tundra (Zamin et al., 2014), Brazilian Cerrado communities (Lannes et al., 2016) and Canadian peatland (Wang et al., 2016).

These variable responses may result from differences in life-form and/or species-level nutrient requirements and differences in physiological capacities (Townsend et al., 2007). For example, studies in European calcareous grasslands have suggested that grasses are N-limited, while legumes are P-limited, owing to their N-fixing ability (Willems et al., 1993; Stöcklin and Körner, 1999; Köhler et al., 2001). In our study, forbs and shrubs, which had higher N contents and N:P ratios than grasses, were also mainly limited by P. Apart from the general patterns of differences in nutrient concentrations among functional groups (Aerts, 1996; Han et al., 2011), this may have resulted in part from the fact that the forb and shrub groups include some leguminous species, such as *I. pseudotinctoria* and *L. corniculatus*. Moreover, shrubs, which are more massive than grasses and forbs, require larger amounts of these nutrients to produce structural and photosynthetic tissues, and showed greater biomass responses to multiple nutrient supplement in our sterile habitats.

As indicated by our relative biomass values, P and K addition favored a shift in the abundance of shrubs relative to grasses, especially under the NPK treatment, suggesting that fertilization was not only increasing the productivity of karst grassland but also altering community composition. Because the NPK treatment favored the growth of

shrubs, shrubs showed an increasing growth and biomass accumulation over time compared to grasses and forbs. In addition, many shrubs, such as *R. parvifolius* and *R. cymosa*, are typically much taller than the grass and forb species and form closed canopies, which may suppress the growth of the herbaceous species. Similar results have been reported in other calcareous habitats (Niinemets and Kull, 2005). To a certain extent, our finding provides direct support for the conclusion of Zhang et al. (2015) that the grasslands are mainly limited by N and shrublands are co-limited by N and P or other nutrients during secondary karst vegetation succession.

#### 4.3. Implications for vegetation restoration in karst habitats

Vegetation reconstruction and ecosystem restoration in karst areas is extremely difficult and is a slow and lengthy process (Guo et al., 2011; Jiang et al., 2014). Many reforestation practices seem not to be successful (Pan et al., 2015) due to the great variability of environment, combined with nutrient and water deficiencies (Du et al., 2011), and/or by a lack of knowledge about how these stresses limit plant growth and vegetation recovery. Our study clearly demonstrates that degraded karst grassland is co-limited by N, P and K and that different plant functional groups are limited by different elements. Mineral fertilization would be a good measure to promote vegetation recovery in the karst region (Zhang and Wang, 2009). Because soil nutrient status and the nutrient requirements of plant communities largely vary between different karst habitats and vegetation types (or recovery stages), further studies are needed to elucidate how nutrients limit the growth of vegetation at different successional stages on different karst landforms, including types of limiting elements and the degree of limitation associated with each element. Moreover, a previous study has indicated that deciduous trees show a high level of dominance in karst forests due to their good adaptation to drought stress (Liu et al., 2010, 2011) and significantly contribute to nutrient cycling of ecosystems by producing lots of leaf litter and promoting the decomposition of the litter layer (Liu et al., 2016). As the total amount of nutrients is very limited in the karst soils, the recycling of essential elements through litter decomposition seems to be particularly important for the nutrient budget of karst ecosystems. Many deciduous dominant and common species, especially leguminous species, could be suitable candidates for reforestation programs in the karst regions.

#### 5. Conclusions

This study demonstrated that the productivity of degraded karst grassland is co-limited by N, P and K, with N and P being the primary limiting factors and K the secondary factor. Among functional groups, grasses are mainly limited by N and P, forbs by P, and shrubs by all three elements, with P being the most limiting factor. Fertilization with multiple elements could help accelerate the succession of secondary grassland to shrubland, speeding recovery of vegetation from rock desertification in this karst region. Because drought stress is another important factor limiting vegetation recovery in this region, further long term studies with regard to the effects of supplemental nutrients and water are needed to examine the interaction effects of the two main limiting factors on the productivity of karst vegetation at different successional stages.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2018.04.008>.

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