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Variation in Leaf Nitrogen and Phosphorus Stoichiometry in Different Functional Groups of Legumes

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ABSTRACT

The legume is notable owing to their symbiotic nitrogen (N) fixing ability. Usually, higher leaf N concentration and N to phosphorus (P) ratio (N:P) in legumes than non-legumes. However, the variations of leaf N, P and N:P and their relationship had been hardly studied based on functional groups. In this study, we studied the leaf N, P and N:P and their relationship among different functional groups. The results showed that the average values of leaf N, P and N:P ratios for all legumes were 27.33 mg g^{-1}, 1.27 mg g^{-1} and 21.94, respectively. Leaf N (36.96 mg g^{-1}) and P (2.15 mg g^{-1}) of herbaceous legumes are significantly higher than N (24.97 mg g^{-1}) and P (1.18 mg g^{-1}) in woody plants, respectively. Moreover, leaf N, P and N:P of shrub markedly higher than them in tree. Leaf N and P are always higher in deciduous than evergreen legumes. A negative correlation was found between leaf N:P and P in overall and different functional groups of legumes.

Key words: Functional groups, Leaf N, Leaf N:P, Leaf P, Leaf stoichiometry, Legumes.

INTRODUCTION

Fabaceae, as the third-largest land plant family, distributes widely all kinds of ecosystems. Legumes are notable for their symbiotic nitrogen (N) fixing ability, which result in higher N concentration and N to phosphorus (P) ratio (N:P) and more N-homeostatic than non-N_2-fixing plants (Güsewell *et al.*, 2003; Guo *et al.*, 2017).

It is well known that the cycle of carbon (C), N, P and their interaction plays key role in driving ecosystem processes (Aerts and Chapin, 2000). Recently, a particular focus of ecological stoichiometry is used to study ecosystem processes (Castellanos *et al.*, 2018). There is a growing recognition that plant functional groups are important controllers of ecosystem processes and functions, which lead to the attention in leaf stoichiometric traits based on different functional groups (Castellanos *et al.*, 2018; Shi *et al.*, 2012, 2017).

N and P usually influence ecosystem function and dynamics due to their limitation in some ecosystems (Sterner and Elser, 2002). The patterns of N, P and N:P in different spatial scales or plant functional groups have been increasingly studied (Han *et al.*, 2005; He *et al.*, 2006a, b, 2008; Lu *et al.*, 2016; Pawar *et al.*, 2003; Reich and Oleksyn, 2004; Shi *et al.*, 2020). He *et al.* (2008) showed leaf N, P and N:P are different among grass, herb and woody plants. Castellanos *et al.* (2018) also drawn similar conclusion based on different functional groups. Meanwhile, the differences of leaf traits between legumes and non-legumes have been focused because of the symbiotic N-fixing strategy of legumes (Guo *et al.*, 2017). He *et al.* (2008) also suggested that legumes had higher N:P than non-legumes. The similar finding was also testified in other ecosystems, such as Sonoran Desert (Castellanos *et al.*, 2018), tropics (Bhaskar *et al.*, 2016; Nasto *et al.*, 2014), wetlands (Güsewell *et al.*, 2003), the karst region (Pan *et al.*, 2015) etc.

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Although the higher leaf N concentration and N:P in legumes than non-legumes had been confirmed, the variations of leaf N, P and N:P and their relationship among different functional groups within Fabaceae are not any reported in our knowledge. In this study, we compared the leaf N, P, N:P and their relationship among different functional groups based on 467 samples within Fabaceae. Our objectives were to: (1) document the patterns of leaf N, P and N:P stoichiometry in different functional groups; and (2) determine the synergistic variation of leaf N and P within legumes.

MATERIALS AND METHODS

Data collection

The data derive from 5 published papers and our collecting samples, which include 467 leaf data in legumes belonging

to 207 species The data of 138, 112, 56, 58 and 11 data were obtained from Guo *et al.* (2017), Han *et al.* (2005), He *et al.* (2008, 2006b) and Wright *et al.* (2004), respectively. Further, we collected 92 samples from wild legumes in China. All leaf samples were oven-dried at 78°C. Dried samples were ground to a fine powder using a ball mill. Leaf N and P were analyzed using an elemental analyzer and the molybdate/ascorbic acid method after H_2SO_4-H_2O_2 digestion (Jones, 2001). We established a legume dataset of leaf N, P and N:P.

Data analysis

The data were subjected to one-way analysis of variance using the SPSS software package version 19.0 (SPSS, Chicago, IL). All leaf traits were normalized by logarithmic transformation prior to statistical analysis. The frequency distributions of leaf N, P and N:P were analyzed.

RESULTS AND DISCUSSION

Patterns of Leaf N, P and N:P across all legumes

For all species, the average values of leaf N, P and N:P were 27.33, 1.27 mg g^{-1} and 21.94, respectively (Table 1). Leaf N in this study is significant higher than previous studies with 20.6, 20.2, 20.1and 19.3 mg g^{-1} respectively (Elser *et al.*, 2000; Han *et al.*, 2005; Reich and Oleksyn, 2004; Wright *et al.*, 2004). However, our finding is accord with legume species reported in different ecosystems or biomes with 25.63, 26.7, 35.8 and 28.2 mg g^{-1}, respectively (Castellanos *et al.*, 2018; Guo *et al.*, 2017; He *et al.*, 2006b; Wright *et al.*, 2004). The reasons of higher leaf N in legumes than non-legumes may be caused by improving N supply in legumes because N addition enhanced leaf N (Jiang *et al.*, 2019).

Leaf P in this study is consistent with legume shrub made by Guo *et al.* (2017) with 1.27 mg g^{-1} (Table 1, Fig 1 and Fig 2). However, our result of leaf P is lower compared with previous majority reports either in legumes or non-legumes. The leaf P of legumes was 2.38 mg g^{-1} for woody legumes in Southeast China (Wu *et al.*, 2012), 1.9 mg g^{-1} in Chinese grassland biomes (He *et al.*, 2008), 1.45 in the Sonoran Desert (Castellanos *et al.*, 2018). The low leaf P could be related with N nutrient improvement because N addition decreased leaf P (Jiang *et al.*, 2019). The ratio (21.94) of N and P in this study (Table 1) is higher than non-legumes, which have been confirmed (Castellanos *et al.*, 2018; Guo *et al.*, 2017; He *et al.*, 2008). This should be lead to by leaf high N and low P owing to N improvement.

Effect of Functional Groups on Leaf N, P and N:P of legumes

When the different functional groups were considered, we found that leaf N and P of herbaceous legumes with 36.96 and 2.15 mg g^{-1} are higher significantly than those of woody legumes with 24.97 and 1.18 mg g^{-1}, respectively (Table 1, Fig 1). However, the N:P of herbaceous is lower than that in woody legumes. Further, the concentration of leaf N and P and N:P are markedly higher in shrub than tree (Table 1, Fig 2), which presented the similar results in overall woody, shrub and tree (Table 1, Fig 2). The higher leaf N and P and lower N:P always exist in deciduous comparing to evergreen legumes (Table 1, Fig 2).

The differences of leaf N, P and N:P among functional groups of legumes in this study supported previous findings (Han *et al.*, 2005; He *et al.*, 2006a,b, 2008; Liu *et al.*, 2019). Our finding in leaf N is consistent with previous studies (He *et al.*, 2006a,b) and inconsistent with existing reports (Castellanos *et al.*, 2018, Wang *et al.*, 2015) when the herbaceous and woody legumes are considered. Further, leaf P and N:P in this study are inconsistent with existing reports (Castellanos *et al.*, 2018, He *et al.*, 2008; Wang *et al.*, 2015). While deciduous and evergreen legumes are compared, the trends of leaf N, P and N:P in this study are accord with previous report made by Han et al, 2005. Certainly, besides the effect of functional groups, it is inevitable that environmental factor maybe also affect leaf trait (Chen *et al.*, 2015; Kirnak *et al.*, 2016; Zhou *et al.*, 2019), which need be explored further.

Table 1: Leaf N, P and N:P ratios for different function groups of legumes.

Group	Leaf N (mg g^{-1})				Leaf P (mg g^{-1})				Leaf N:P			
	n	mean	SD	CV	n	mean	SD	CV	n	mean	SD	CV
Overall	400	27.33	10.24	0.37	362	1.27	0.65	0.52	296	21.94	9.67	0.44
Herb	79	36.96**(a)	10.17	0.28	32	2.15**(a)	0.71	0.33	32	17.72(b)	5.05	0.29
Woody	321	24.97	8.77	0.35	330	1.18	0.58	0.49	264	22.45**	9.97	0.44
Woody:shrub	201	27.15**(b)	7.58	0.28	186	1.29**(b)	0.53	0.41	185	23.26*(a)	9.90	0.43
Woody:tree	120	21.30(c)	9.42	0.44	144	1.04(c)	0.61	0.59	79	20.57(ab)	9.94	0.48
Woody:deciduous	209	36.61**	7.54	0.21	216	1.38**	0.56	0.41	191	20.35	5.99	0.29
Woody:evergreen	92	18.84	8.12	0.43	104	0.76	0.38	0.50	63	28.68**	15.75	0.55
Shrub:deciduous	158	27.87**	7.11	0.26	152	1.39**	0.49	0.35	151	20.97	5.73	0.27
Shrub:evergreen	32	21.61	6.84	0.32	27	0.78	0.49	0.63	27	35.26**	17.49	0.50
Tree:deciduous	51	24.15**	8.19	0.34	64	1.35**	0.71	0.52	40	18.01	6.45	0.36
Tree:evergreen	60	17.36	8.41	0.48	77	0.76	0.34	0.45	36	23.75*	12.40	0.52

n, number of samples; mean value, SD, coefficient of variation (CV, defined as SD/mean) are reported. Differences between each group were tested using a one-way ANOVA; significant differences at $P < 0.05$ are indicated by asterisk or different letters.

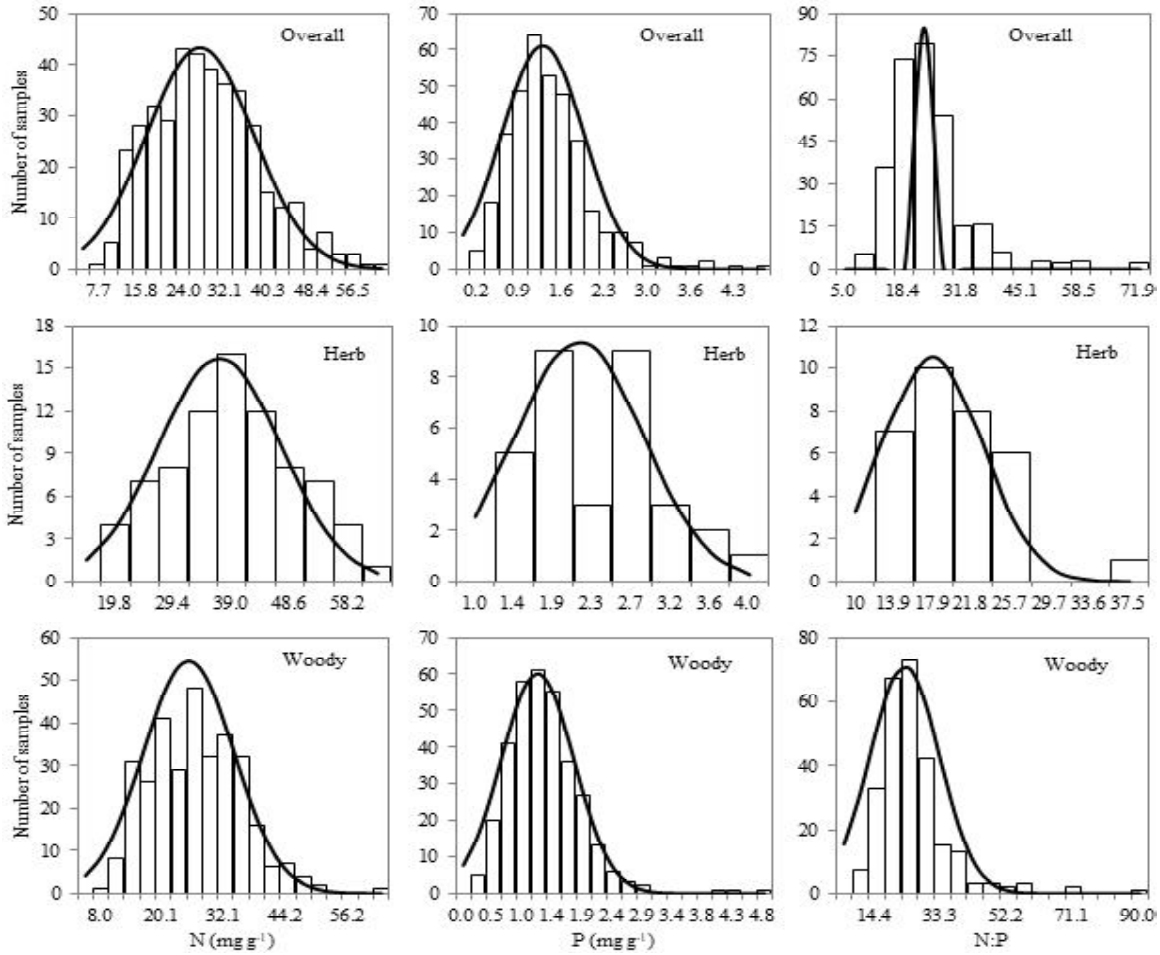


Fig 1: Histogram distribution of leaf nitrogen, phosphorus and N:P ratio in different function groups of legumes.

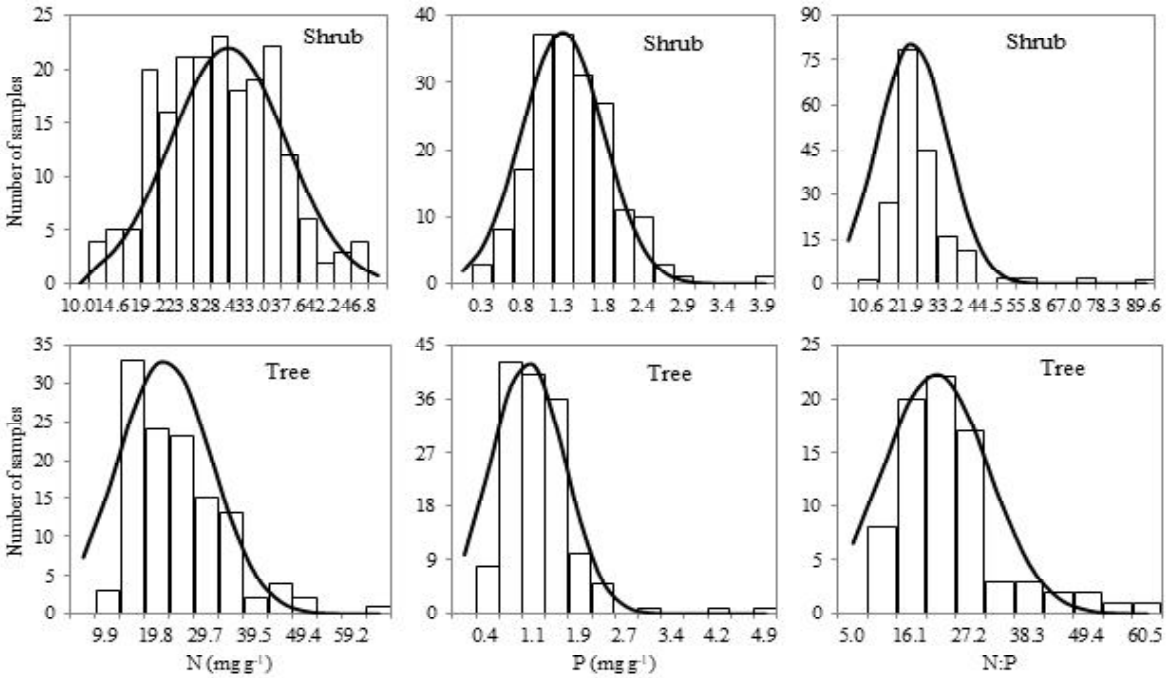


Fig 2: Histogram distribution of leaf nitrogen, phosphorus and N:P ratio in shrub and tree of legumes.

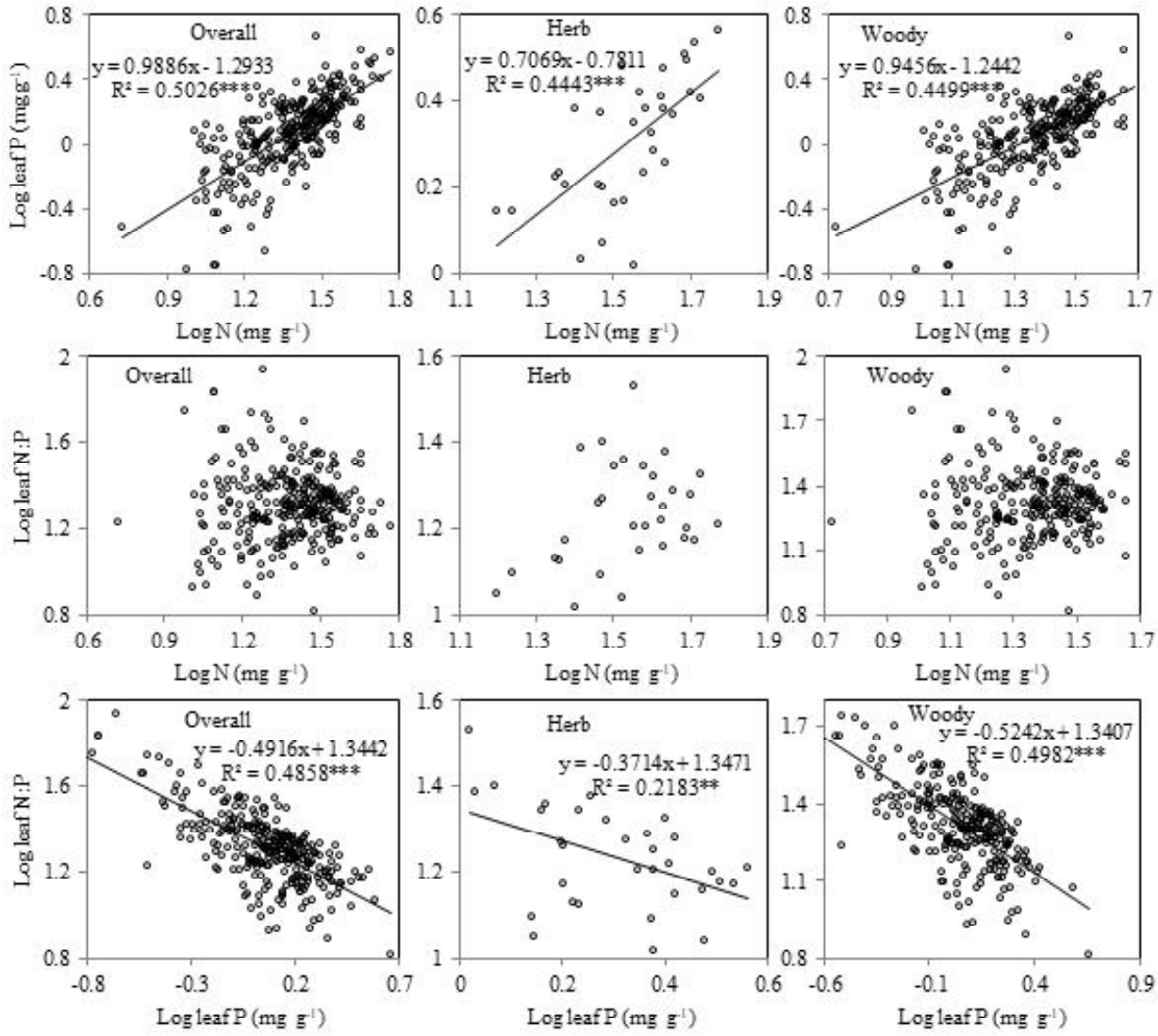


Fig 3: Relationships between leaf nitrogen, phosphorus and the mass ratio of N and P of different functional groups in legumes.

Leaf N, P and N:P Relationships of legumes

Leaf P increased significantly with the enhancement of leaf N in overall, herbaceous, woody, shrub and tree legumes ($p<0.001$) (Fig 3). Leaf N explained 38.87% to 50.26% of total variations of leaf P in all functional groups of legumes (Fig 3, Fig 4). As far as the relationship between leaf N:P and leaf N or P are concerned, no any relationship is observed between leaf N:P and leaf N in any functional groups. However, leaf N:P decreases significantly with the increase of leaf P in all functional groups (Fig 3, Fig 4). Leaf P explain 48.58% of the variation of leaf N:P in overall legumes (Fig 4). As to herbaceous and woody legumes, leaf N:P was greater explained by leaf P changes in woody than herbs (Fig 2). When the woody was further considered, leaf P variation was accounted for more changes of leaf N:P in shrub than tree legume group (Fig 4).

The present study demonstrated that leaf N and P were highly positively correlated, which is in agreement with previous studies (Castellanos *et al.*, 2018; Guo *et al.*, 2017; Güsewell, 2004; He *et al.*, 2008; Wang *et al.*, 2015). While a negative correlation was found between leaf N:P and P (Fig 3, Fig 4) in overall and different functional groups of legumes, which have been testified that the increase of leaf P lead to the enhancement of leaf N:P (Jiang *et al.*, 2019). The finding of relationship between leaf N:P and N was contrary with previous conclusion that leaf N:P increase generally with the enhancement of leaf N (Pan *et al.*, 2015; Wang *et al.*, 2015). The relationship between leaf N:P and N or P made in Chinese grassland biomes and Chinese sea-buckthorn supported our conclusion (He *et al.*, 2008). It is recognized that leaf N:P is an indicator of nutrient limitation (Aerts and Chapin 2000; Güsewell, 2004; Wassen *et al.* 1995). P is restrictive when leaf N:P ratio of more than 16 (Wassen *et al.* 1995). The leaf N of legumes was much higher than that non-legumes (Table 1), while the high N:P ratio suggested that P limits plant growth.

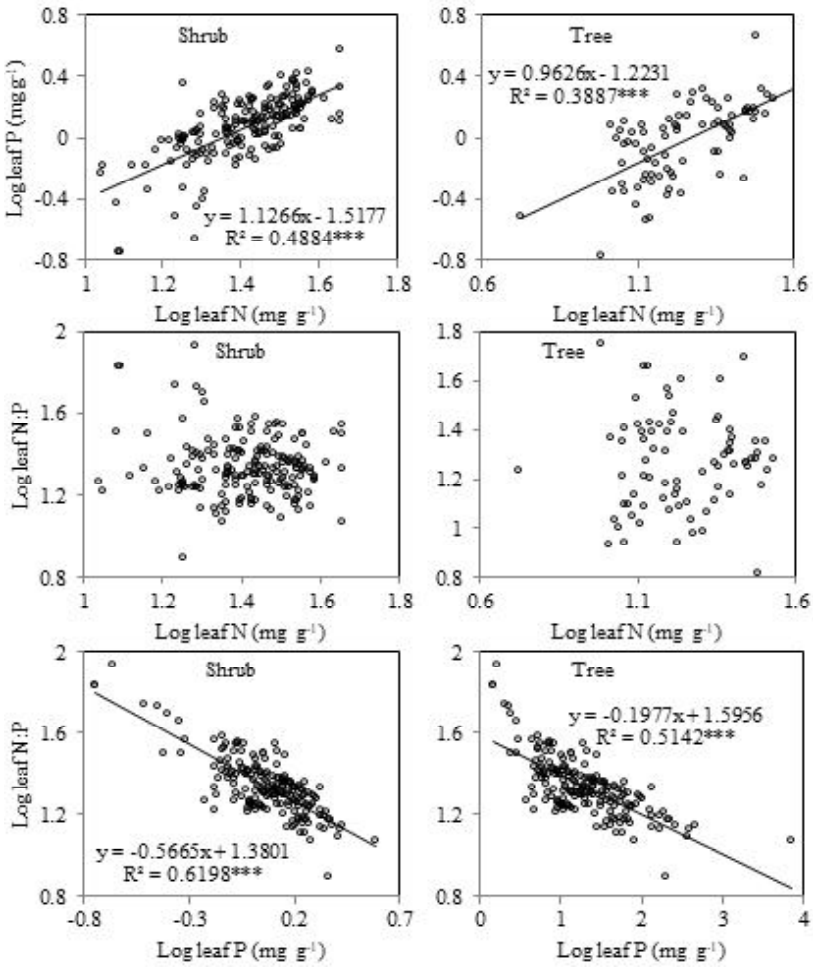


Fig 4: Relationships between leaf nitrogen, phosphorus and the mass ratio of N and P of shrub and tree in legumes.

CONCLUSION

The average values of leaf N, P and N:P ratios for all legumes were 27.33, 1.27 mg g^{-1} and 21.94, respectively. Leaf N and P of herbaceous legumes are significantly higher than N and P in woody plants, respectively. Moreover, leaf N, P and N:P of shrub markedly higher than them in tree. Leaf N, P and N:P always present the same trend when the deciduous and evergreen woody legumes are compared regardless of shrub or tree. Leaf N and P are positive correlative in overall, herbaceous and woody legumes. A negative correlation was found between leaf N:P and P in overall and different functional groups of legumes.

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