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Leaf Nitrogen and Phosphorus Stoichiometry are Closely Linked with Mycorrhizal Type Traits of Legume Species

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ABSTRACT

Arbuscular mycorrhizas (AM) are the most widely symbiosis in terrestrial ecosystem. Leaf N and P are the most important plant functional traits due to their influence on biogeochemical cycling. However, as the notable nitrogen (N) fixing plants, the variations of leaf N, P and N:P and their relationship among mycorrhizal types had been hardly studied based on functional groups. In this study, we studied the leaf N, P and N:P and their relationship between AM and other mycorrhizal types (others) among different functional groups or climate zones. The results indicated that AM improve significantly leaf N and P, while reduce N:P comparing to others. However, the influences of AM and others on leaf N, P and N:P changed with plant functional groups or climate zone. The relationships between leaf N and P, between N and N:P and between P and N:P also exhibit great variation between AM and Others.

Key words: Arbuscular mycorrhizas, Functional groups, Leaf N, Leaf N:P, Leaf P, Legumes, Mycorrhizal type.

INTRODUCTION

Legumes are characterized as maintaining stable nutrient supply under nutrient-limited conditions due to their types to acquire nitrogen (N) from the atmosphere by symbiotic nitrogen fixation. Usually, the capacity of N fixation of legume plants lead to higher N concentration, the ratio of N and phosphorus (P) and more N-homeostatic than non-N-fixing plants (Guo *et al*., 2017; Shi *et al*., 2020a). This may be a reason for the wide distribution of legume species in all kinds of ecosystems.

Leaf N, P and N:P are vital in holding ecosystem functioning and dynamics (Kerkhoff *et al*., 2006; Vitousek *et al*., 2010). Leaf N regulates the processes of photosynthesis, plant production and litter decomposition (Lebauer and Treseder, 2008), while P take responsibility for energy storage and tissue structure. Although leaf N coordinated with P in assimilating carbon and transpiration (Kerkhoff *et al*., 2006), their variation exist in different plant functional groups, e.g. between herbaceous and woody plants (Shi *et al*., 2020b), between deciduous and evergreen species (Kerkhoff *et al*., 2006), between legumes and non-legumes (Guo *et al*., 2017) and among different mycorrhizal types (Shi *et al*., 2020a).

Mycorrhizas are the widest symbiosis forming between plant root and soil mycorrhizal fungi in terrestrial ecosystem (Smith and Read, 2008). Mycorrhizal status of plants is the most typical belowground traits (Heijden *et al*., 2015), which help host plants acquire soil resources and adapting habitats in their evolution (Ma *et al*., 2018; Shi *et al*., 2017). Mycorrhizas produce direct or indirect impacts on leaf trait (Reich, 2014). Further, the various functions of different mycorrhizal types in all kinds of ecosystems have been reported (Averill *et al*., 2014; Shi *et al*., 2012). As the most abundant mycorrhizal association, arbuscular mycorrhizas (AM) facilitate hosts to uptake mineral nutrients and improve the nutritional status of host plants, including P and N (Smith and Read, 2008).

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The effects of different mycorrhizal types on leaf trait have been widely reported, but there are not inconsistent conclusion, probably because of various plant phylogeny, growth form and habitat. Jespersen *et al*. (2019) found leaf traits co-vary with AM features during plant succession. Koele *et al*. (2012) reported no consistent impact of ectomycorrhizas on leaf traits. Our previous study indicated that AM affected the leaf traits and their responses to precipitation and temperature (Shi *et al*., 2012). However, the influence of different mycorrhizal types on legumes remains unknown.

In the present study, the legume leaf traits of N, P and N:P in solo AM and other mycorrhizal types (others) were compared and the interrelationships among leaf N, P and N:P were analyzed. Our aims are to test how to effect of different mycorrhizal types of AM and others on leaf N, P and N:P among different legume functional groups and climate zones.

MATERIALS AND METHODS

Data collection

We draw out the legume leaf N, P and N:P data from a global database of paired leaf nitrogen and phosphorus

concentrations of terrestrial plants established by Tian *et al*. (2019). The 1021 leaf data in legumes were obtained belonging to 300 species and 141 plant genus. On this basis, a new database was established including leaf characteristics and mycorrhizal type according to the mycorrhizal types of legume plants. The database was finished in Henan University of Science and Technology in 2019. The mycorrhizal type of each plant species was ascertained according to the published literature, especially Harley and Harleye (1990), Koele *et al*. (2012), Shi *et al*. (2020a), Wang and Qiu (2006). We classified all the legume plants with solo AM as AM type and other mycorrhizal types (others), such as ectomycorrhizas, ERM and simultaneous two or more mycorrhizal typles as others type. Further, legumes were subdivided into two subgroups based on their growth forms including herbs, shrubs and trees. According to the leaf trait, legumes were also classified into deciduous and evergreen broadleaved species.

Data analysis

All leaf traits were normalized by logarithmic transformation prior to statistical analysis. The permutation tests were performed with 9999 permutations for comparing leaf N, P and N:P of AM and others groups by R 3.6.0 (http://R-project.org/). Data are presented as mean ± standard error. The frequency distributions and boxplot of leaf traits were analyzed by the SPSS software package version 19.0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Leaf N, P and N:P between AM and Others among functinal groups and climate zones

For all 1021 legume samples, permutation test results indicated that the significant variations existed between AM and others for legume leaf N, P and N:P (Fig 1 and 2). According to growth form of legumes, leaf N and P of AM plants are always markedly higher than them in others species with the exception of leaf P in herbs. When the classification of deciduous or evergreen was considered, deciduous broadleaved N and evergreen broadleaved P in AM groups were remarkable higher than them in others group. Compared to others, AM species had significant lower N:P in functional groups of shrub, tree and evergreen legumes. Further, leaf N of AM species is always higher than others either tropical or temperate zones (Fig 2). Leaf P of AM group is also higher than others in tropical zone.

Here we first evaluated the relationship between different mycorrhizal type (AM and others) and legume leaf N and P traits. It has been confirmed that the different mycorrhizal types lead to the variation of host N, P and other mineral nutrients (Averill *et al*., 2019; Shi *et al*., 2013, 2020b). Usually, AM is better at enhancing absorption of mineral nutrients and improving photosynthetic pigments and water status (Smith and Read, 2008), which could explain partly the higher leaf N and P in AM than others mycorrhizal plants. The lower N:P of AM plants than others be caused possibly

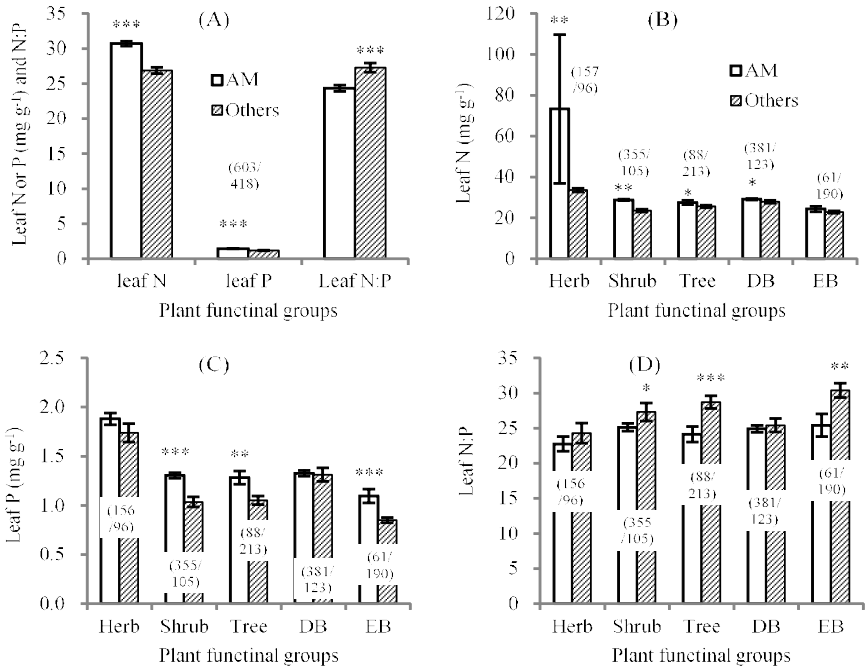


Fig 1: Leaf nitrogen (N), phosphorus (P) and the mass ratio of N and P (N:P) (mean ± SE) of different legume plant groups with mycorrhizal types of AM and others. The number in the columns represents the amount of species in group of AM/others, respectively. DB means deciduous broadleaved species, EB means evergreen broadleaved species. The asterisks *, ** and *** above the bars represents significant differences between mycorrhizal types AM and others at P < 0.05, P<0.01 and P<0.001 respectively, based on the permutation tests using log_{10}-transformed values. The same is below.

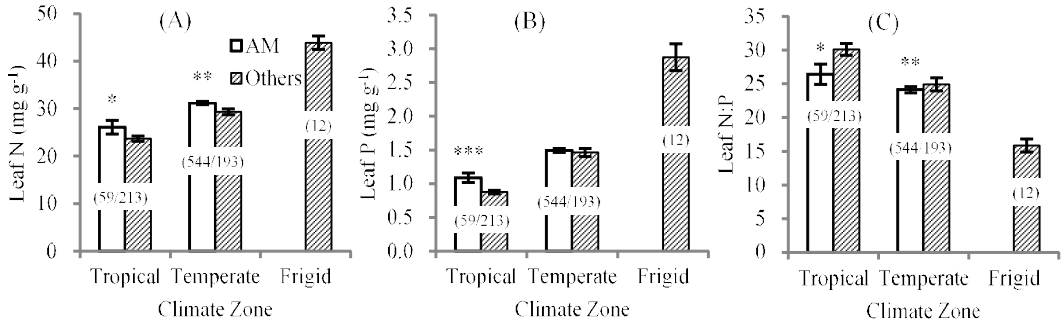


Fig 2: Leaf nitrogen (N), phosphorus (P) and the mass ratio of N and P (N:P) (mean ± SE) of legume plant groups with mycorrhizal types of AM and others in different climate zones.

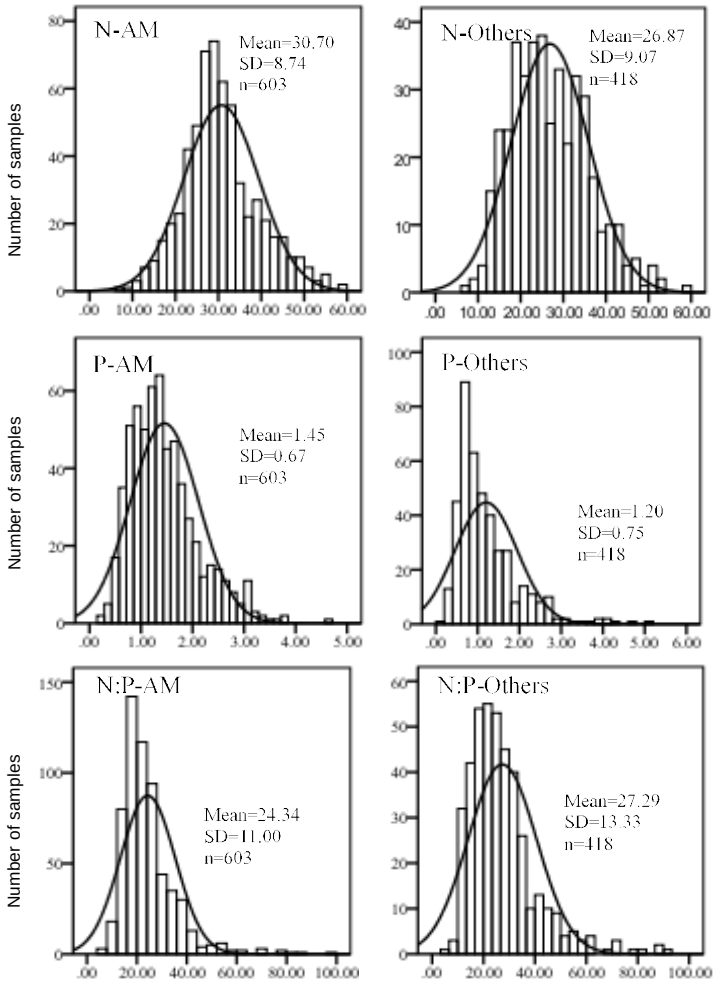


Fig 3: Histogram distribution of leaf N, P and N:P in plant groups with mycorrhizal types of AM and others.

by discrepant uptake of mineral nutrients among mycorrhizal types (Averill *et al*., 2019; Cornelissen *et al*. 2001). Another possible reason is that the AM is better at improving N-fixing efficiency for legumes than others because the interaction between AM and N-fixation has been testified (Kalkal *et al*., 2018; Seyahjani *et al*., 2020). Certainly, the different responses of AM and others species among different functional groups could be explained by the different plant growth forms and different tissue types (Averill *et al*., 2019; Shi *et al*., 2020a). The findings of mycorrhizal stretegies on leaf N and P among different climate zones supported the conclusion made by Averill *et al*. (2019), which are possibly leaded by plant evolution traits, environments and their interaction (Brzostek *et al*., 2017).

Distribution of leaf N, P and N:P between AM and others

Leaf N, P and N:P varied greatly for both AM and others legume species, but the ranges were trifle wider for N and

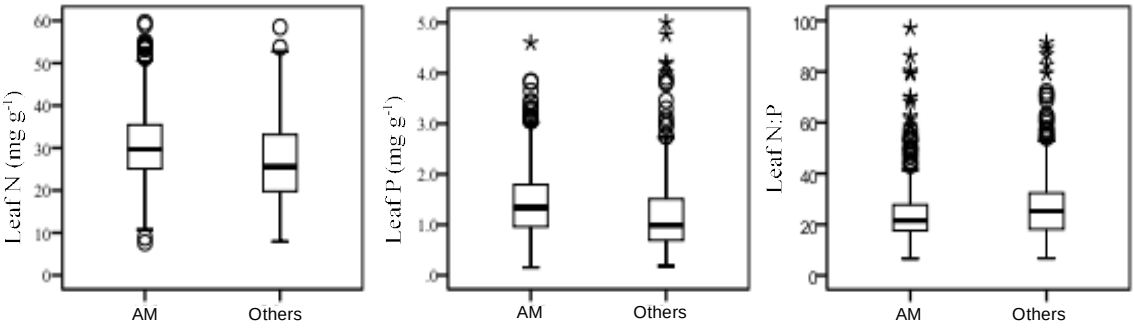


Fig 4: Box plot of legume leaf N, P and N:P in groups with mycorrhizal types of AM and others.

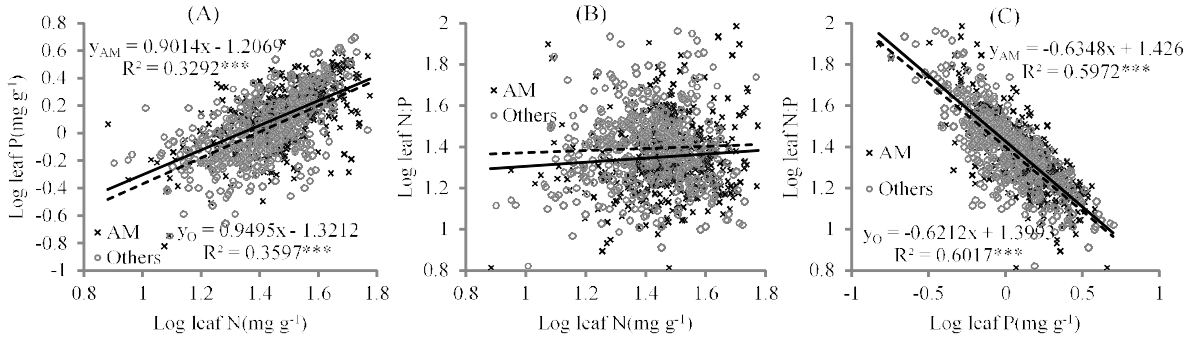


Fig 5: Relationships between leaf N, P and N:P in legume plant groups with mycorrhizal types of AM and Others. The solid and dash regression lines mean the mycorrhizal types of AM and others, respectively. The same is below.

N:P in AM species and for P in Others (Fig 3 and 4). Leaf N ranged from 7.63 to 59.60 mg g^{-1} with the mean of 30.70 mg g^{-1} in AM species, while from 7.98 to 58.50 mg g^{-1} with the average of 26.87 for Others plants (Fig 3). Leaf P changed from 0.15 to 4.60 and 0.18 to 5.00 mg g^{-1} with the average of 1.45 and 1.20 mg g^{-1} for AM and Others, respectively. N:P ranged from 6.54 to 97.12 for AM and from 6.63 to 91.40 for others species, respectively. The box-plot indicated further that AM plants had significantly higher N and P and lower N:P than others (Fig 4).

The larger range of leaf N and N:P in AM plants consistent with previous reports (Shi *et al*., 2020a), which may be explained by the wide distribution or higher diversity of AM plant in whole terrestrial ecosystems comparing to others (Cosme *et al*., 2018). Around 60% samples are AM species in our study, which lower than traditional recognition in 71% in vascular plants (Brundrett, 2017) because we considered only the solo AM as AM type in the current study. Probably, the wider range of leaf P in others than AM group is caused by the multi-mycorrhizal types. Certainly, the ranges of N, P and N:P should be related with plant evolution and adaptation traits, which need to be explored.

Leaf N, P and N:P relationships

When all legumes were considered, leaf P increased significantly with the enhancement of leaf N in AM and Others plants (Fig 5). Leaf N explained 32.92% and 35.97 % P variations for AM and Others, respectively. Leaf N:P was not affected by the changes leaf N, while decreased significantly with N increase for either AM or others in overall 1021 legumes. As to different functional groups, the relationship between any two leaf traits, the responses for AM and others groups were different in most situations (Fig 6 and 7). Larger changes of leaf P with increase of leaf N existed in others species than AM but tree and evergreen broadleaved legumes. The responses of N:P to N changes were more sensitive with the higher explanation in others than AM among all plant groups with the exception of herb group. The explained degree of P to N:P changes always lower in AM than Others plants for herb, shrub, tree, deciduous and evergreen broadleaved legumes (Fig 6 and 7). The positive relationship between leaf N and P supported previous finding (Castellanos *et al*., 2018; Guo *et al*., 2017), while their varied correlation between AM and others with different plant functional groups may be led by different responses of P to N improvement due to different mycorrhizal functions (Averill *et al*., 2019; Cornelissen *et al*. 2001). For example, the relationship between N and P showed that the same N-change caused the more change of P in others than AM plants. That is to say, the P changes in others groups are more sensitive than in AM. This maybe owes to much improvement of N in others plants, which accelerate the P accumulation (Jiang *et al*., 2019). The systematic differences in AM and Others could be explained by the conclusion of AM vs. EM (Ectomycorrhiza) associated with nutrient acquisitive vs. nutrient conservative plant economic traits, respectively (Averill *et al*., 2019) because the others group including EM and other mycorrhizal types. However, the variation of leaf traits with different mycorrhizal types cannot be attributed to shared evolutionary history coupled with

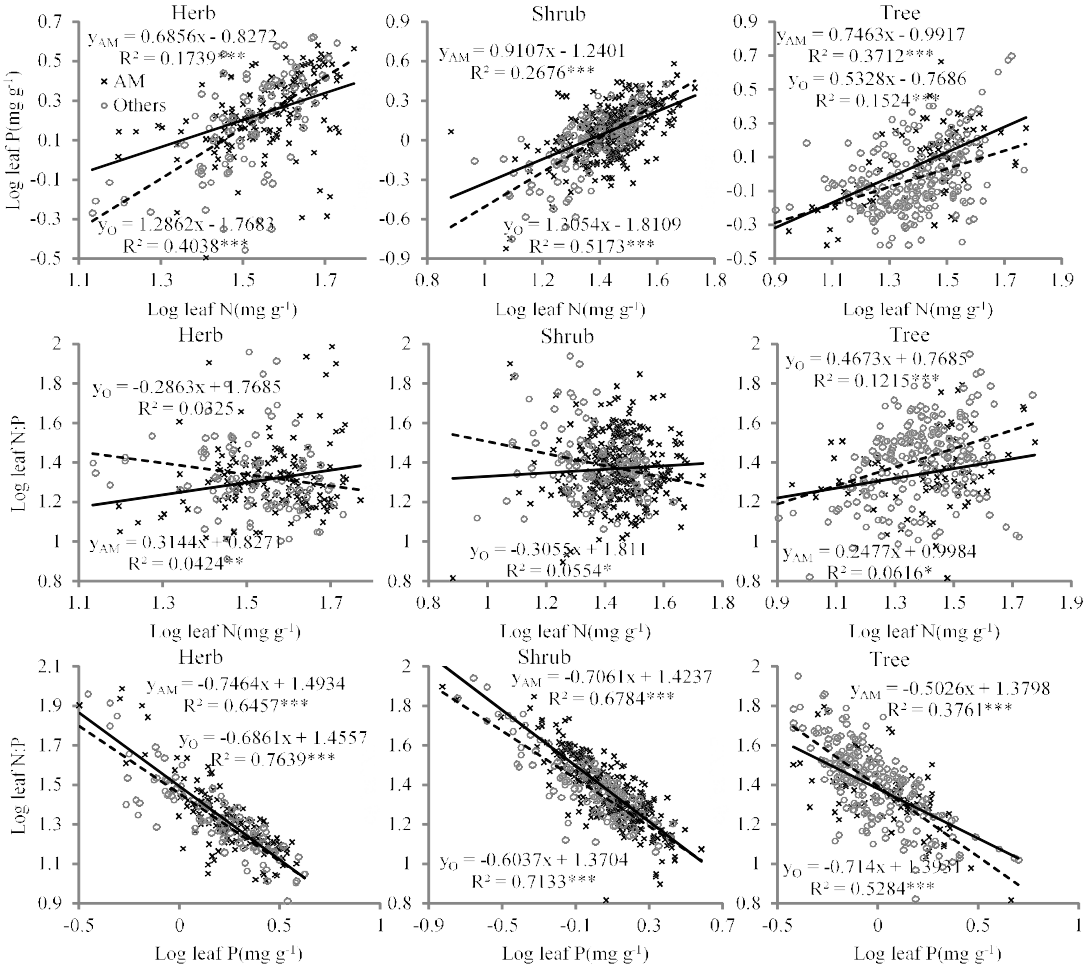


Fig 6: Relationships between legume leaf N, P and N:P in mycorrhizal types of AM and others among herbaceous, shrub and tree legume plants.

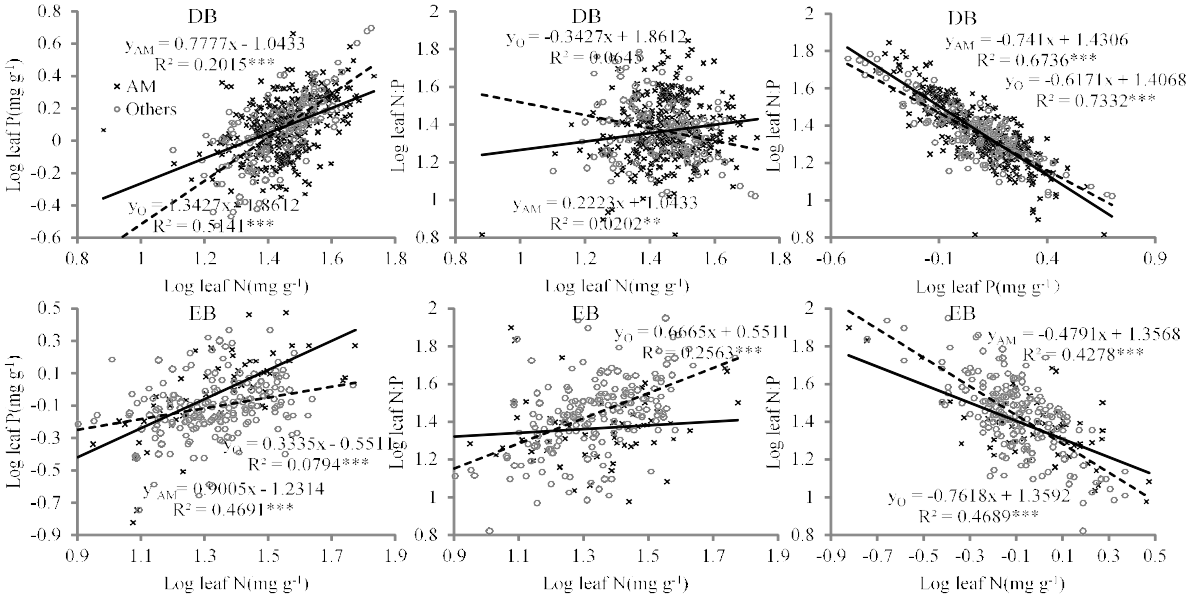


Fig 7: Relationships between legume leaf N, P and N:P in mycorrhizal types of AM and Others among deciduous broadleaved (DB) and evergreen broadleaved (EB) legume species.

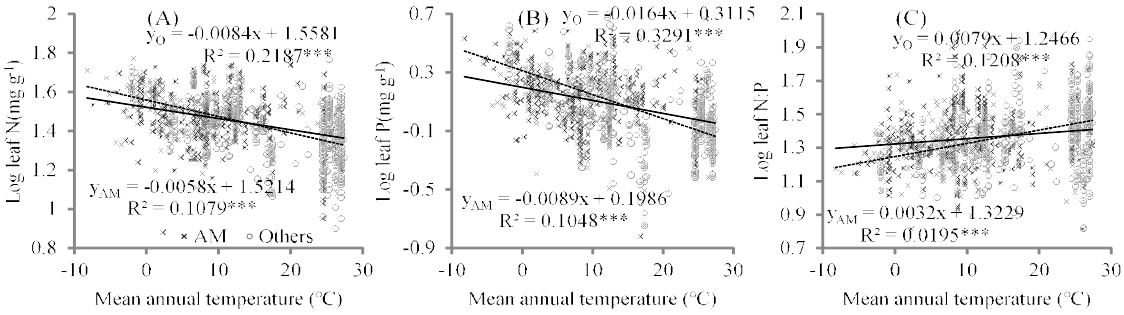


Fig 8: The changes of legume leaf N (A), P(B) and N:P(C) with mean annual temperature in mycorrhizal types of AM and others.

historical oversampling of particular plant clades (Averill *et al.*, 2019). Therefore, the further studies are necessary for exploring the possible mechanisms of the variable responses of leaf traits to different mycorrhizal types among different plant functional groups.

Effect of mean annual temperature on Leaf N, P and N:P between AM and others

The changes of leaf N, P and N:P of legume with mean annual temperature (MAT) between AM and others were presented in Fig 8. The responses of leaf N, P and N:P to changes of MAT in AM species are less sensitive than in others. The similar conclusion that varied responses of leaf N, P and N:P among different mycorrhizal types had reported based on the grassland species (Shi *et al.*, 2013). Certainly, the detailed reasons need to be further explored.

CONCLUSION

Legume leaf N, P and N:P are closely linked with mycorrhizal type traits. AM improves significantly leaf N and P concentration, while decreases leaf N:P. The influences of mycorrhizal types on leaf N, P and N:P change with different plant functional groups or climate zone. Although the relationships between leaf N and P, between N and N:P and between P and N:P are consistent between AM and Others among all legumes, they also exhibit great variation with the differences of mycorrhizal types. Leaf traits of AM species are less sensitive to MAT changes than others plants.

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