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Original Research

Difference in Phosphorus Acquisition Strategies of N_2-Fixing Plants in Shrubland and Primary Forest Soils of the Karst Regions

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Abstract

Phosphorus (P) acquisition strategies of plants to adapt to P limitation in primary forest ecosystems of karst regions remain unclear. Root phosphatase and arbuscular mycorrhizal (AM) fungi, involved in two common P acquisition strategies, were measured in two N_2-fixing plants (*Albiziaodoratissima* and *Cajanuscajan*) growing in shrubland and primary forest soils with and without soil AM inoculation. Both plants cultivated in primary forest soils had lower AM colonisation and N_2 fixation rates but higher root acid phosphatase activity in the rhizosphere when compared with those in shrubland soils. Plants in shrubland soils predominantly exploit P resources by stimulating AM colonisation of roots, but do so by enhancing root phosphatase activity in primary forest soils. AM colonisation in both N_2-fixing plants was positively correlated with N_2 fixation rates but negatively correlated with root acid phosphatase activity. Soil available P content was higher in both N_2-fixing plants under soil inoculation with AM fungi than in the treatment without fungal inoculation; root and shoot P content did not vary significantly between treatments. AM fungi and phosphatase enzymes increase N_2-fixing plants' capacity to obtain soil P, thus contributing to the decrease in soil P limitation in karst ecosystems.

Keywords: karst ecosystem, nutrient acquisition strategies, phosphorus limitation, *Arbuscular mycorrhizal* fungi, phosphatase

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Introduction

Phosphorus (P) is an essential nutrient that limits plant production and performance in natural ecosystems [1-3]. Although the total amount of P in soils often exceeds the demand of plants, approximately 20% of P (in form of orthophosphate) is available for plant uptake [4]. Hence, plants are generally limited by low P availability in terrestrial ecosystems [5, 6], especially in forest ecosystems [1]. A similar situation also occurs in karst regions [7, 8], and seems to be more serious because high soil calcium causes immobilization of plant-available phosphorus, forming different calcium phosphate compounds. For example, a previous study carried on karst regions has indicated that plants are limited by soil P, and P deficiency is more evident in a later vegetation restoration stage (i.e., primary forest) than in an early vegetation restoration stage [7].

Plants have a degree of plasticity in surviving limiting environments and have evolved various strategies to overcome soil P limitation [1, 9, 10]. Colonisation with arbuscular mycorrhiza (AM) and synthesis of phosphatase enzymes are two P acquisition strategies vital for plant growth. For example, phosphatase can mineralize more organic P from ester-bound forms to orthophosphate form and increase plant P uptake [11]. Thus, high phosphatase activity is often detected on or around plant roots when soil P availability is low [12]. Alternatively, 80% of terrestrial plants form symbiotic associations with AM [13]. AM fungi can enhance access to orthophosphate pool through hyphae or secreted phosphatase which increases plant P uptake [14]. Therefore, increase in the phosphatase activity and the degree of AM colonisation could provide plants with an advantage of P acquisition in soils that are characterized by P limitation.

AM colonisation and root phosphatase activity of plants, which were simultaneously measured in different soil P levels, showed that plants in low P soils had a higher rate of phosphatase activity and AM colonisation than plants in high P soils [15]. However, the variation in AM colonisation is much greater than the variation in root phosphatase activity among different soil P levels [15]. Simultaneously, a previous research indicated that a relatively small increase in AM colonisation is needed for achieving rates of P uptake similar to that achievable with a relatively large increase in phosphatase enzymatic activity [16]. Furthermore, some studies suggested that early-successional plant species acquire P mainly by enhancing phosphatase activity, whereas late-successional species absorb P mainly through increasing AM colonisation [17]. Liu et al. [18] also recorded higher diversity of AM fungi in the late-successional ecosystems compared to that in the early-successional ecosystems. If these observations were broadly considered in plant growth in different vegetation restoration stages with different soil P levels (lower lever of P content in shrubland but higher lever of P content in primary forest), it would imply that plants growing on primary forest soils can rely on AM fungi to acquire P, while in shrubland soils, plants can strongly rely on phosphatase synthesis to increase P uptake. As a consequence of these differences, plants have evolved different strategies to acquire soil P and to further promote vegetation restoration.

N_2-fixing plants are widely used for vegetation restoration in karst regions or/and other regions due to their greater contribution to acquire nitrogen and phosphorus for plant. The ability of N_2-fixing plants for fixing N_2 is well known, however, whether they could acquire soil phosphorus through N_2 fixation is poor known. The previous studies indicate that N_2 fixation could enhance photosynthesis [19], allowing for relatively greater C investment in AM colonisation and secreted phosphatase, which can then promote plant acquisition of soil phosphorus. Our previous study indicated that AM colonisation with roots were significantly influenced by plant functional groups in the karst regions [20]. Simultaneously, soil AM fungal communities varied from grassland to primary forest, and *Glomus* dominated in these sites of the karst regions [21]. Therefore, in this study, we conducted a potted experiment with two N_2-fixing plants (*Albiziaodoratissima* and *Cajanuscajan*), which are the native trees in karst regions [22], and widely distributed in shrubland and primary forest ecosystem. The individuals of N_2-fixing plants cultivated in shrubland soils and primary forest soils were examined for any variations in acquisition strategies of soil P (i.e., AM colonisation and root phosphatase activity). In addition, the individuals of N_2-fixing plant were cultivated in both soils with sterilization (without inoculation of soil with AM) and without sterilization (inoculation of soil with AM) and were used to assess the effect of AM colonisation on soil P acquirement of plants. We hypothesised that both investigated N_2-fixing plants would predominantly exploit P by enhancing root phosphatase activity in shrubland soils, but in the forest soils, they would predominantly exploit P via stimulating AM colonisation of roots.

Materials and Methods

Experimental Design

We conducted a potted experiment in Huanjiang Observation and Research Station for Karst Ecosystems of the Chinese Academy of Sciences, located in southwestern of China (24°44'N, 107°51'E). This region has a subtropical mountainous monsoon climate. The mean annual rainfall is 1,389 mm and the mean annual air temperature is 8.5°C. This region experiences a wet season from April to August, accounting for 70% of the annual precipitation [21].

Soils for the potted experiment were collected from shrub ecosystem (24°44'N, 107°48'E) and primary forest ecosystem (25°06'N, 107°53'E) located in the

surroundings of Huanjiang Observation and Research Station. These soils are classified as calcareous lithosols (limestone soil) according to the FAO/UNESCO classification system [23]. Soils were sieved (5 mm) and stored in dark at 4ºC. The levels of total N and available P in shrubland soils were 4.48 g kg^{-1} and 1.92 mg kg^{-1}, respectively. The levels of total N and available P in the primary soil were 7.35 g $kg^{-1}$and 4.31 mg kg^{-1}, respectively.

The experiment was set up in a random factorial design with three factors:(1) N_2-fixing plant species, including *A. odoratissima* and *C. cajan*, which are widespread and abundant species of symbiotic fixers in karst ecosystems (the detailed information for both N_2-fixing plants are shown in Table 1); (2) soil type, including shrub ecosystem soil and primary ecosystem soil; (3) two levels of AM fungi, AM inoculation or not. Plots filled with all of non-sterilised soils indicated inoculated AM fungi. However, plots filled with all of sterilised soils indicated non-inoculated AM due to soil sterilised kill AM. Soils were sterilised in an autoclave oven for 120 min at 120ºC [24]. To ensure the effectiveness of the sterilization procedure and plants without-inoculated AM, AM colonisation was determined in the sterilized soils after plant harvest. Five replicates for each treatment were carried out and a total of 40 samples were collected.

Seeds of *A. odoratissima*and *C. cajan* were collected nearby the study site, sterilized for 1 min in 10% household bleach solution, washed with distilled water, and sown in 200 ml plastic cups in homogenized sterilized soil. Two seedlings per species with similar growth potential were planted in each pot (depth 30 cm, diameter 30 cm) three weeks after sowing. Only one seedling with better growth potential was in a pot one week after transplantation (Fig. 1). Plants were watered gravimetrically by weighing each plot every second or third day. 15 seedlings per species were harvested in order to measure initial biomass (ranging from 0.13 g in *A. odoratissima* to 0.51 g in *C. cajan*; Table 1).

Sampling and Analysing

The pot experiment began at May 6. 2018, after 15 weeks, all plants were harvested. Plants were divided for the harvest of leaves, stems, and roots. Roots were washed with deionized water. One part was stored in 95% ethanol for analysis of AM colonisation, and another part was stored at -20ºC for analysis of root phosphatase activity. The remaining roots, leaves, and stems were dried at 65ºC for 48 h and weighed in order to measure plant biomass and tissue C:N:P ratio. Total plant dry biomass was used to calculate relative growth rate (RGR) as follows:

$$RGR = (\log_e(Mf) - \log_e(Mi))/dt$$

...where M_i is the initial mass, M_f is the final mass, and d_t is the duration of the experiment in days [1].

During harvest, the rhizosphere soil was also sampled. One portion of rhizosphere soil was air-dried for analysis of available phosphate (AP), while the other portion was stored at 4ºC for analysis of soil phosphatase activity.

Fig. 1. Experiment photographs.

Table 1. Two N_2-fixing plant species used in the experimental studies organized by family, functional group, initial mass and geographic distribution (data came from Flora ReipublicaePopularisSinioae, http://frps.iplant.cn).

Species	Family	Functional group	Distribution	Initial mass (g)
Albiziaodoratissima Benth	Albizia	N_2 fixer	Southwest China	0.13
Cajanus cajan Millsp	Cajanus	N_2 fixer	Southwest and southeast China	0.51

N fixation rates were measured using acetylene reduction assay method [25] (ARA). During the harvest of N_2-fixing plants, fresh nodules were excised from roots and placed in a conical flask (125 ml). The conical flask was sealed and 10% acetylene atmosphere was injected, and afterwards it was incubated for 30 min *in situ*. 30 ml gas samples were removed from the sealed conical flask after incubation, injected in a 12 ml pre-evacuated glass vial (LabcoExetainer, Labco Limited, UK), and transferred into a laboratory for analysis with a gas chromatograph (Agilent GC 7890A, Agilent, USA) equipped with a flame ionization detector (FID).

Acid phosphatase (phosphomonoesterase) activity in root was measured according to Png et al. [26]. The buffer used was 50 mM sodium bicarbonate (pH 5.0). The 12 wells were assigned into a sample assay (0.8 ml buffer + 20~30 g roots + 200 µl 200 µM MUB-linked substrate), roots control (1 ml buffer + 20~30 g roots), negative control (0.8 ml buffer+200 µl 200 µM MUB-linked substrate), reference standard (0.8 ml buffer+200 µl 10 µM 4-MUB solution), quench standard (0.8 ml buffer + 20~30g roots + 200 µl10 µM 4-MUB solution), and blank wells (1 ml buffer). These 12-well plates were shaken at room temperature with 110 rpm for 1 h and the reaction was stopped with 10 ml NaOH (1.0 M). For each replicate, 200 µl subsamples were pipetted into a black 96-well microplate. Acid phosphatase activity was determined using a microplatefluorometer (Infinite 200 Pro, Tecan, Switzerland) at 365 nm excitation and 450 nm emission. Root phosphatase activities were calculated as µmol 4-4-MUB-P g^{-1} root h^{-1}.

AM colonisation was measured as described in Zhang [24]. Fine roots (1 cm in diameter) from each sample were randomly selected and root AM colonisation was quantified using the magnified intersection method. A total of 300 intersections of each root sample were taken.

Statistical Analyses

Data analysis and calculations were performed using the SPSS statistical software (SPSS Inc., Chicago, IL). The homogeneity of variance was tested prior to the statistical analysis. First, the differences of biomass, RGR, AM colonisation, root phosphatase activity, and N_2 fixation soil AP, RGR, shoot P, and root P between shrubland soil and primary forest soil as well as between soil inoculated with AM fungi and soil without inoculation with AM fungi were presented them as bar graphs (Fig. 2, 4), and these differences were estimated by linear mixed effects models (lme4 package with R). In these models, plant species, soil types and AM fungi inoculation were modelled as fixed factors, and two sampling sites were modelled as random factors (Table 2). Afterwards, the Pearson's correlation analysis was used to determine the relationship between root phosphatase activity (RPA), AM colonisation, and N_2 fixation (Fig. 3). Finally, we calculated the relationships between the AM colonisation, RPA, RGR, soil P content, shoot P content, and root P contents, and presented them as scatter plots (Fig. 5).

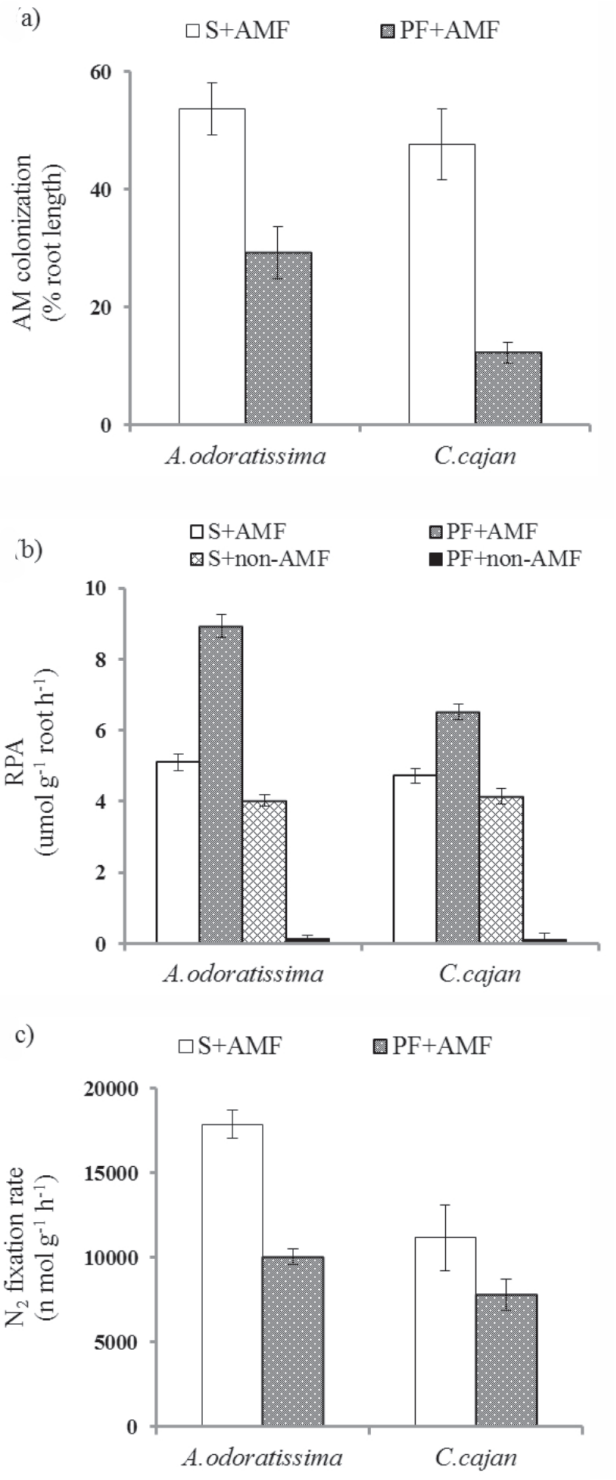

Fig. 2. AM colonization a), root phosphatase activity (RPA; b) and N_2 fixation c) response of two N_2-fixing plants in shrubland and primary forest soils inoculation soil AM fungi or non-AM fungi (S, shrubland; PF, primary forest; AMF, inoculated with arbuscular mycorrhizal fungi; non-AMF, not inoculated arbuscular mycorrhizal fungi).

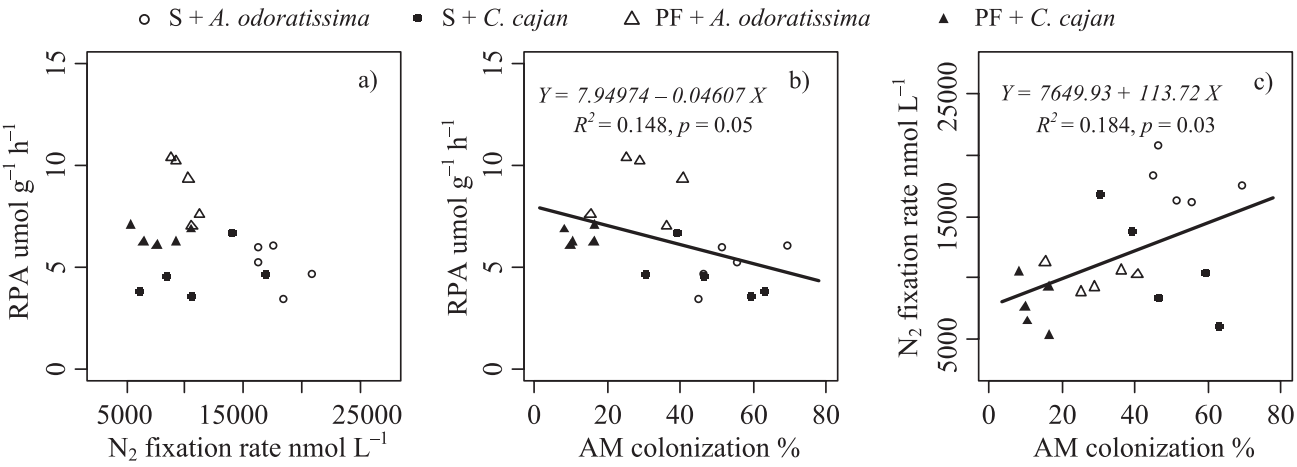

Fig. 3. Relationships among N_2 fixation, root phosphatase activity (RPA) and AM colonization of two N_2-fixing plants in shrubland and primary forest soils (S, shrubland; PF, primary forest; AMF, inoculated with arbuscular mycorrhizal fungi; non-AMF, not inoculated arbuscular mycorrhizal fungi).

Results

Mycorrhizal Colonisation, Phosphatases, and N_2 Fixation Rate

AM colonisation rates of *A. odoratissima* cultivated in shrubland soils and primary forest soils were 53% and 29%, respectively. AM colonisation rates of *C. cajan* cultivated in shrubland soils and primary forest soils were 47% and 12%, respectively (Fig. 2a). Hence, AM colonisation levels of both investigated N-fixing plants cultivated in shrubland soils were higher than those in primary forest soils (Fig. 2a), and N_2 fixation rate was similar to AM colonisation rate (Fig. 2c).

Root phosphatase activities of *A. odoratissima* and *C. cajan* cultivated in primary forest soils were significantly higher than those cultivated in shrubland soils when they were inoculated with AM fungi.

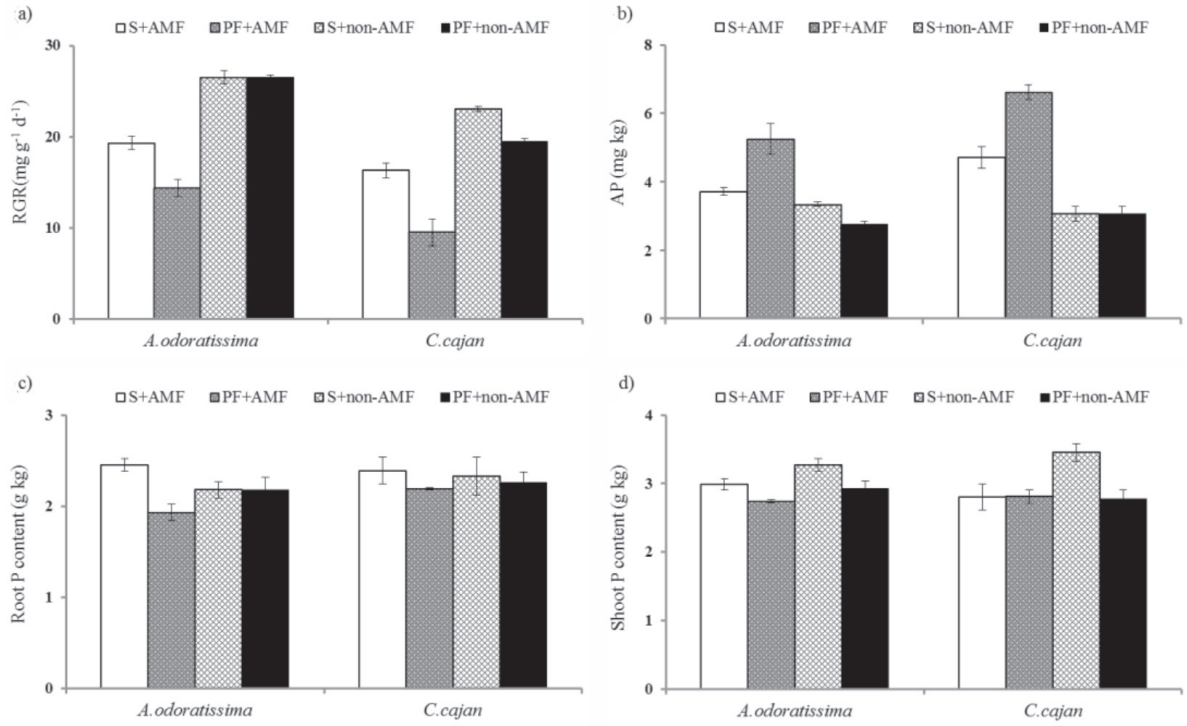

Fig. 4. RGR a), AP b), root P content c) and shoot P content d) response of two N_2-fixing plant species in shrub and primary forest soils inoculation soil AM fungi or non-AMF (S, shrubland; PF, primary forest; AMF, inoculated with arbuscular mycorrhizal fungi; non-AMF, not inoculated arbuscular mycorrhizal fungi).

Additionally, the RPAs of *A.odoratissima* and *C.cajan* in AM-inoculated soil were higher than those in uninoculated primary forest and shrubland soils (Fig. 2b).

AM colonisation was negatively correlated with RPA ($p = 0.05$; Fig. 3b), while was positively correlated with N_2 fixation ($p = 0.03$; Fig. 3c).

Plant Growth, Soil AP, and Root and Shoot P Contents

Whole plant biomass and relative growth rate differed between different soil types (Table 2) as well as between soils inoculated and uninoculated with AM fungi (Fig. 4). Relative growth rates and root P content of both *A.odoratissima* and *C.cajan* were higher in AM-colonised shrubland soils compared to those in primary forest soils (Fig. 4(a-c)), while the trend of soil AP content in these plants rhizosphere was opposite (Fig. 4b). The shoot P content of *A.odoratissima* in AM-colonised shrubland soils was higher compared to that in AM-colonised primary forest soils, while in *C. cajan* no significant differences were found between two soils (Fig. 4d). The soil AP content in *A. odoratissima* and *C. cajan* in AM-colonised soil were higher compared to those in uninoculated soil (Fig. 4b), while this trend was reversed for RGR (Fig. 4a).

Effect of Mycorrhizal Colonisation and Phosphatases on Plant Growth, Soil AP, and Root and Shoot P Contents

Plant growth, soil AP, and root and shoot P content responded differently or interactively to different plant species, soil types, inoculated, and uninoculated soil (Table 2). The ratio of AM colonisation to root phosphatase activity (AM colonisation: RPA) was positively correlated with RGR of both plants ($p = 0.0003$, Fig. 5a), while it was negatively correlated with soil AP content ($p = 0.0003$, Fig. 5b). However, AM colonisation: RPA was not correlated with shoot and root P content (Fig. 5(c-d)).

Discussion

Difference in P Acquisition Strategies of N_2-fixing Plants in Shrubland and Primary Forest Ecosystems

In this study, plants growing on shrubland soils had the highest rate of AM colonisation and lowest root phosphatase activity. Additionally, AM colonisation to root phosphatase activity ratio was positively correlated with RGR. These results suggested that plants growing on shrubland soils mainly depend on AM colonisation for acquiring P. By contrast, plants growing on primary forest soils had the highest root phosphatase activity but the lowest rate of AM colonisation, and AM colonisation to root phosphatase activity ratio was negatively correlated with RGR. This indicated that plants growing on primary forest soils rely on phosphatase for acquiring P. Thus, we concluded that the N_2-fixing plants have unique and dominant strategies of soil P acquisition, allowing them to perform better in different vegetation restoration stages.

There are several possible explanations why N_2-fixing plant would predominantly exploit P by enhancing AM colonisation in shrubland soils but do so via stimulating root phosphatase activity in primary forest soils. First, AM fungi are well known to promote P acquisition by plants [13]. Our previous study has shown that soil AM fungal taxa differ from grassland to primary forest and diversity of soil AM fungal taxa decrease with increasing vegetation succession in karst region [21]. This might indicate that some AM fungal taxa disappeared during succession from shrubland soils to primary forest soils, resulting in plants relying less on AM to acquire P in primary forest soils. This is further confirmed by our present results showing that N_2-fixing plants had lower rates of AM colonisation in primary forest soils when compared with those in shrubland soils. However, plants have a certain degree of plasticity and exploit other acquisition strategies (e.g., phosphatases) in order to absorb P when AM colonisation is low [1]. This is in agreement with our results that showed N_2-fixing plants had higher root phosphatase activity in primary forest soils when compared with shrubland soils (Fig. 2b). It is important to note that a previous study reported that AM colonisation is more efficient in promoting P acquisition by plants compared to phosphatase [16]. Therefore, some AM fungal taxa disappeared in the primary soil which led to a low rate of AM colonisation. The explanation for this was that plant growth is more restricted by soil P in the primary forest ecosystems compared to other ecosystems of karst regions [7, 8]. Second, the possible resource-driven relationship between AM colonisation and N_2 fixation could also explain this phenomenon. N_2 fixation can increase the photosynthetic rate of N_2-fixing plants, possibly allowing them to invest more C to support greater AM colonisation [15]. However, in the present study, N_2-fixing plants had lower N_2 fixation rates in the primary soils, which led to a low rate of AM colonization (Fig. 1c), and a decrease in P acquisition (Fig. 4a). Although the question whether N_2 fixation is necessary to acquire P or vice versa remains, a positive correlation between N_2 fixation rates and AM colonisation in this research provides evidence for a link between P acquisition and N_2 fixation in karst ecosystems (Fig. 3c). Third, AM colonisation of roots was correlated with soil P content. Although AM fungi had an advantage in acquiring inorganic P [1, 13], the level of AM colonisation was inhibited by high soil P content. Thus, higher soil P content in the primary forest soil led to a lower level of AM colonisation in the primary forest soil compared to that in the shrubland soil.

Table 2. Effect of soil type, plant species and soil AM fungi addition on the soil AP, RGR, Shoot P, and Root P.

Response variable	Linear mixed models	AIC	BIC	*T* value	Significant Difference
Soil AP	Null	140.36	145.42	16.05	a
	Soil type	140.72	147.48	9.89	a
	Species	138.41	145.17	11.54	b
	AMF addition	97.52	104.28	6.86	**c**
	Soil type * Species	138.61	147.05	8.18	b
	Soil type * AMF addition	92.94	101.38	18.19	**d**
	Species * AMF addition	83.81	92.26	5.95	**e**
	Species * Soil type * AMF addition	78.36	88.49	17.57	**f**
RGR	Null	259.03	264.10	10.13	a
	Soil type	257.29	264.04	9.14	b
	Species	253.69	260.44	10.42	c
	AMF addition	229.60	236.35	26.37	**e**
	Soil type * Species	251.56	260.00	10.29	d
	Soil type * AMF addition	230.25	238.69	12.90	**e**
	Species * AMF addition	214.31	222.75	24.59	f
	Species * Soil type * AMF addition	214.21	224.34	16.59	**f**
Shoot P	Null	27.52	32.59	18.96	a
	Soil type	24.12	30.87	40.95	d
	Species	29.46	36.21	18.19	a
	AMF addition	28.44	35.20	21.74	a
	Soil type * Species	26.05	34.50	5.05	b
	Soil type * AMF addition	25.80	34.25	36.19	c
	Species * AMF addition	30.38	38.82	20.57	a
	Species * Soil type * AMF addition	27.74	37.87	4.64	a
Root P	Null	21.95	27.01		a
	Soil type	20.24	26.99		b
	Species	22.46	29.22		a
	AMF addition	19.74	26.49		c
	Soil type * Species	20.68	29.12		b
	Soil type * AMF addition	20.58	29.03		b
	Species * AMF addition	20.16	28.61		b
	Species * Soil type * AMF addition	20.96	31.10		b

Null model: $f\mathrm{i} = \alpha + \mathrm{bi} \times Z \text{ sites} + \varepsilon\mathrm{i}$; (2) Soil type or Species or AM fungi addition model: $f\mathrm{i} = \alpha + \beta \times X$ (Soil type or Species or AM fungi addition) + $\mathrm{bi} \times Z$ sites + $\varepsilon\mathrm{i}$; (3) Soil type * Species * AMF addition model: $f\mathrm{i} = \alpha + \beta 1 \times X$ soil type + $\beta 2 \times X$ species + $\beta 3 \times X$ AM fungi addition + $\mathrm{bi} \times Z$ sites + $\varepsilon\mathrm{i}$. Here, $f\mathrm{i}$ is the response variable, X is the fixed effect, Z is the random effect, α is the intercept of model, β is the parameter of fixed factor, bi is the parameter of random factor, and $\varepsilon\mathrm{i}$ the unexplained information. The optimal model was defined with minimum AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion). In these models, soil type, species and AM fungi addition were mixed random factors, sampling site were random factor. The random factor was merely captured by the null model. Different letters in the last column indicate instances when the mixed model was better than the null model when mixed factors soil type, or species, or AM fungi were captured by the model.

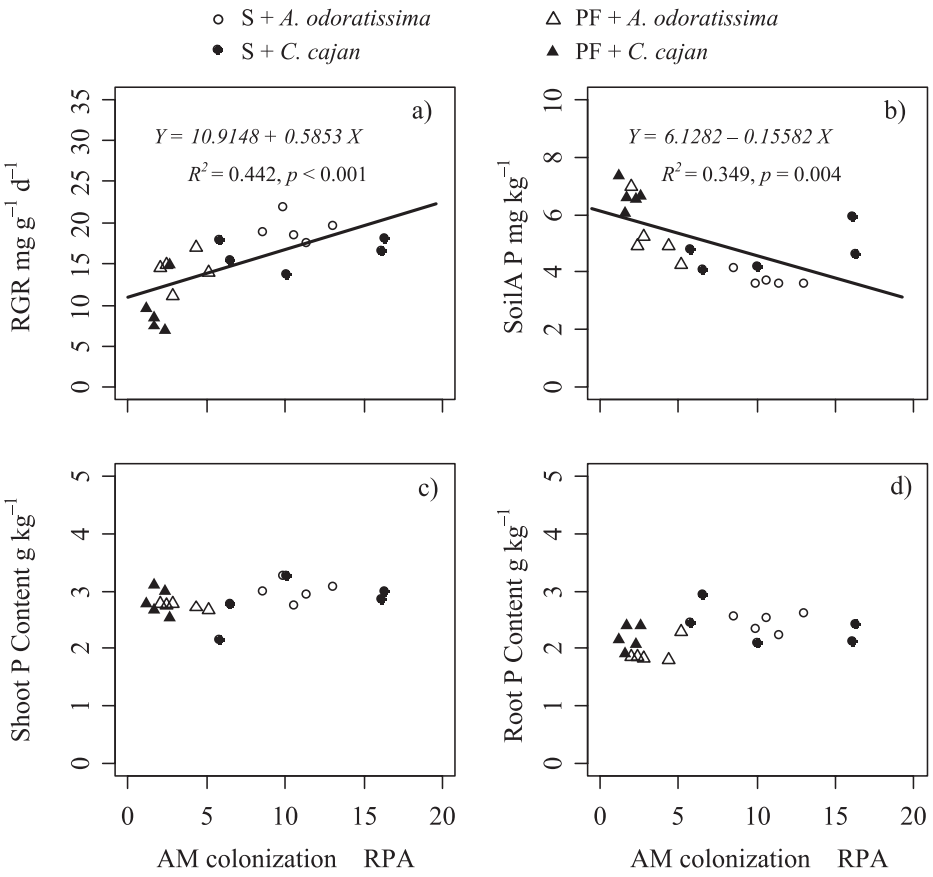

Fig. 5. Relationship between the arbuscular mycorrhzal colonization to root phosphatase activities (AM colonization: RPA), relative growth rates (RGR), soil P content, shoot P content, and root P content (S, shrubland; PF, primary forest; AMF, inoculated with arbuscular mycorrhizal fungi; non-AMF, not inoculated arbuscular mycorrhizal fungi).

Role of AM Fungi on P Acquisition in Plants of Karst Ecosystems

Many studies have demonstrated that plants have the capacity to acquire more P through increasing the AM colonisation and phosphatase enzymatic activity [1, 16]. Our results revealed a negative relationship between AM colonisation and root phosphatase activity (Fig. 3b). The complementarity between AM fungi and root phosphatase in P nutrient foraging may explain these results. Each P nutrient acquisition strategy by plants costs a significant resource [16, 19]. For example, the cost of P acquisition through AM colonisation ranges from 200 to 270 g C g^{-1} P [19]. The N cost of soil P acquisition through phosphatase enzymes ranges from 1 to 16 g N g^{-1} P [16]. From a cost–benefit perspective, the most successful plants should use AM fungi and phosphatase to maximize benefits while minimizing costs for soil resource acquisition. Therefore, plant individuals would mostly benefit from the optimal strategy that maintains the balance or complementarity between efficient soil P acquisition and energetic costs needed for this process. Simultaneously, a negative relationship between AM colonisation and phosphatase activity present in karst ecosystems suggested that AM fungi may not simply acquire inorganic P for plant growth and also secrete phosphatase enzymes to exploit organic P pools.

Enhanced P acquisition strategies, such as increasing AM colonisation of roots, led to the content of root P being higher in the shrubland soil compared to the primary forest soil (Fig. 4c). Although different soil P acquisition strategies may be just one of many possible factors explaining our results, they could contribute to AM fungi having a key role for P acquisition in karst ecosystems. Our study, however, found no significant differences in shoot and root P content between the soil inoculated with AM fungi and uninoculated AM fungi (Fig. 4(c-d)), and similar results were reported by Zhang et al. [24]. There are two possible reasons to explain this result. First, the previous study indicated that bulk additions of phosphorous fertilizer to the experimental pots may increase plant P content over a short time [1]. However, in the present study, plant P content was not significantly changed as a consequence of plant growth in low-phosphorous soil and soil without added phosphorous. Second, soils from different vegetation types can harbour different AM fungal taxa [21], which can differ in their functions in plant growth. Thus, the quantification of intraradical AM colonisation alone may not be representative of the true function of AM fungi. It was noteworthy that plants

have not only showed a host-specificity in symbiosis with beneficial microbes [27], but also shared the same associations with pathogens [28, 29]. The pathogens compete with AM fungi for host carbohydrates, which results in the host plant investing fewer carbohydrates in AM fungi, thus affecting plant P absorption and growth.

Conclusions

This study provided an understanding of differences in nutrient acquisition by the same N_2-fixing plants grown in the shrubland and primary forest soils, and differences in P acquisition strategies of N_2-fixing plants in the soils which could remove soil P limitation. Our results showed that N_2-fixing plants in shrubland soils had higher AM colonisation rates in roots but lower root phosphatase activities when compared with primary forest soils, indicating that N_2-fixing plants would predominantly exploit P by stimulating AM in shrubland soils, but in primary forest soils, they would predominantly exploit P by enhancing root phosphatase activity. Furthermore, soil P acquisition rates were positively correlated with N_2 fixation rates, suggesting that N_2-fixing plants may have a greater capacity for acquiring soil P via phosphatase enzymes and AM fungi, thus greatly contributing to the P cycle in karst regions. Based on our observations, the presence of AM fungi and phosphatase enzymes can enhance the capacity of N_2-fixing plants for acquiring soil P in karst ecosystem. However, the ability to N_2 fixation is only one of many factors that can enhance the capacity of plants for acquiring soil P, and these other factors (e.g., light, plant competition, and pathogens) remain to be studied in the further.

Acknowledgment

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Conflicts of Interest

The authors declare no conflict of interest.

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