Contrasting rhizosphere soil nutrient economy of plants associated with arbuscular mycorrhizal and ectomycorrhizal fungi in karst forests

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Abstract

Purpose Plants growing in the soils of karst forests associate with arbuscular mycorrhizae (AM) or ectomycorrhizae (ECM) to acquire nutrients. We researched how these different mycorrhizal associations affect rhizosphere soil nutrient economy in these calcareous soils.

Methods Bulk and rhizosphere soils were sampled beneath 25 AM and 9 ECM plants growing in primary forests at the Puding Karst Critical Zone Observatory. Nutrient contents and potential enzyme activities were analyzed to test the effect of different types of mycorrhizal association on rhizosphere soil nitrogen (N) and phosphorus (P) economies.

Results The contents of nitrate-N and available-P were markedly lower in the rhizospheres of ECM plants compared to AM plants. Ectomycorrhizal plants promoted relatively greater investment in N-acquisition enzymes, in contrast, AM plants caused relatively greater investment in P-acquisition enzymes. The decreased pH in the rhizospheres of AM plants likely promoted the greater P availability.

Conclusion Our results revealed how plants that form contrasting mycorrhizal associations have fundamentally different effects on rhizospheric nutrient economies in the low fertility karst soils of southwest China. Differentiation in N- and P-acquisition capacity of these plants have implications for species coexistence and the high levels of plant biodiversity observed in these forests.

Keywords: Mycorrhizae; Extracellular enzymes; Rhizosphere effect; Calcareous soil; Plant-soil (below-ground) interactions

Introduction

Symbiotic associations with arbuscular mycorrhizae (AM) or ectomycorrhizae (ECM) have evolved as different effective strategies for increasing plant nutrient acquisition (Ma et al. 2018; Tedersoo et al. 2020). For instance, ECM fungi enhance nutrient acquisition by secreting broad target extracellular enzymes for mining nutrients, especially nitrogen (N), from soil organic matter (SOM), while most AM fungi possess narrow enzymatic capabilities and benefit plants by assisting inorganic N and phosphorus (P) supplies (Cheeke et al. 2017; Phillips et al. 2013). Consequently, plants associated with ECM fungi (ECM plants) prevail in soils with poor N availability, while AM plants dominate in relatively P-limited habitats (Tedersoo and Bahram 2019). The resource allocation strategies of soil microorganisms and plants are expected to adjust their physiological metabolism and to invest more in the production of a particular enzyme when the nutrient targeted by that enzyme is limiting (Burns et al. 2013). Accordingly, ECM plants may predominantly rely on their associated fungi for mineralizing N, while AM associations are vital for acquiring P (Steidinger et al. 2019; van der Heijden et al. 2015). However, we currently have limited understanding of exactly how the different nutrient acquiring functions of AM and ECM plants affect the soil economies of N versus P where plants with different mycorrhizal associations coexist. This is especially the case for biodiverse subtropical forests where soil profiles tend not to have substantial nutrient-rich organic horizons, in contrast to temperate forests.

There may exist distinct trait-integrated biogeochemical syndromes in AM and ECM stands as a result of differences in their 'nutrient economies', as the Mycorrhizal Associated Nutrient Economy (MANE) framework proposed by Phillips et al. (2013). Plant-soil systems dominated by AM associations tend to be characterized by 'fast' cycling of inorganic nutrient economy, while ECM plants thrive in conditions of 'slow' cycling of organic nutrient economy (Phillips et al. 2013). The inorganic nutrient economy results from rapid rates of nutrient mineralization owing to the high chemical quality of AM leaf litter, root exudates and mycorrhizal litter. The organic nutrient economy is due to slow decomposition of litter results in a significant fraction of nutrients exist in organic forms (Phillips et al. 2013). Conflicting evidence persists although there is broad acceptance for contrasting patterns of AM versus ECM associated nutrient cycling in temperate forests (Phillips et al. 2013; Zhu et al. 2018b) and rarely in tropical forests (Lin et al. 2018; Waring et al. 2016). For instance, similar inorganic P pools were found in ECM compared with AM dominated soils in temperate forests (Rosling et al. 2016). The decomposition rates of leaf litter differ between AM and ECM tree types in temperate, but not in tropical or subtropical, forests (Keller and Phillips 2019). Furthermore, the majority of current evidence is from studies of acid forest soils with little understanding of the effect of contrasting mycorrhizal types on rhizospheric nutrient economy in calcareous soils.

Soil nutrient availability influences the effects of mycorrhizal plants on soil nutrient cycling. For instance, the differences in net N mineralization rates of soils between ECM and AM plots are reduced as N availability increases (Midgley and Phillips 2016), and poor availability of soil P inhibits the development of mycorrhiza (Breuillin et al. 2010). Studies in temperate forests may not adequately reflect mycorrhizal type differences in karst habitats because temperate forests are generally more limited by N (Vitousek et al. 2010), while the primary forests in karst areas are more P-limited and harbour different plant lineages (Guo et al. 2019; Li et al. 2018; Ni et al. 2015). In addition, studies have largely focused on the analysis of mineral soil from monospecific plots with AM or ECM species in horticulture or mixed-species plots with different proportions of AM versus ECM species (Cheeke et al. 2017; Lin et al. 2018; Phillips et al. 2013). Furthermore, carbon (C) and nutrients can be transferred from one plant to another through fungal mycorrhizal networks in mixed forests (Tedersoo et al. 2020; van der Heijden et al. 2015). This implies that nutrient economies in the rhizosphere soil of AM and ECM plants in mixed-species forests, such as those of the native mixed evergreen and deciduous forest in the southwest Chinese karst, may be different, but this needs further to explore.

The rhizosphere refers to the soil directly adjacent to roots where microbial biomass and potential enzyme activities may be 2 to 20 times greater than those of bulk soil (Kuzyakov and Blagodatskaya 2015). Soil microorganisms and plant roots can increase nutrient availability by secreting extracellular enzymes that catalyze the mineralization of SOM, and liberate bound P by producing H^+ and low molecular weight organic acids that dissolve calcium compounds in the rhizosphere (Burns et al. 2013; Zhu et al. 2018a). Low inorganic nutrient contents caused shifts in enzyme stoichiometry towards greater investment in N and P acquisition in ECM plots relative to AM plots in temperate forests in North America (Cheeke et al. 2017; Phillips et al. 2013), but knowledge of whether similar changes in enzyme production in the rhizosphere soil of contrasting mycorrhizal plants follows the same pattern in subtropical karst forests is unknown. Although mycorrhizal associated plant roots and mycorrhizal fungi are capable of exuding a large variety of different low molecular weight organic acids (Hinsinger et al. 2011; Zhu et al. 2018a), the relative effects on soil pH and P availability are poorly understood. Large proportions of P is precipitated through binding to calcium phosphate in calcareous soils (Vitousek et al. 2010), so acidification may be more effective in karst soils in liberating P. Thus, the extent to which AM and ECM plants differ in their effect on rhizosphere soil nutrient availability through enzyme production and acidification pathways in calcareous soils might be a useful tool to explore nutrient acquisition function of these plants and their function in nutrient cycling in subtropical karst forests.

Bulk and rhizosphere soils and the first order roots of 34 trees and shrubs were sampled in a primary forest site at the

Puding Karst Critical Zone Observatory. The plants selected for sampling were all angiosperms to reduce the influence of plant phylogeny. We researched the effects of contrast mycorrhizae on rhizosphere soil nutrient economy in the same soil type and climate conditions. Our objective was to reveal how AM and ECM plants affect rhizosphere soil nutrient availabilities by regulating enzyme production in order to adapt to the P-limited condition in karst primary forest soils. We hypothesized that (1) mineralized N and available-P contents in the rhizosphere soil of ECM plants were lower than those under AM plants in karst soil, as previously observed in mineral soils (Lin et al. 2018; Phillips et al. 2013); (2) ECM plants and rhizosphere microorganisms secrete more N- and P-acquisition enzymes in response to the reduced N and P availability in the rhizosphere, relative to AM plants (Cheeke et al. 2017); and (3) both ECM and AM plants may increase P availability by decreasing pH and to dissolve calcium phosphate in the rhizosphere to adapt the P-limited condition.

Materials and Methods

Site description

The study site was on Tianlong Mountain (26°14′48″ N, 105°45′51″ E), which is a part of the Puding Karst Critical Zone Observatory (CZO) in southwest China. The primary forest in this region has a typical subtropical monsoon climate, with a mean annual temperature of 15.1 °C and a mean annual precipitation of 1390 mm. The soil is classified as Mollic Inceptisols according to USDA Soil Taxonomy (Soil Survey Staff 2010). Three transects were randomly established onsite from the foot to the top of the mountain in late May 2018. The transects measured 50 m × 10 m and the distance between each transect was about 100 m. Thirty-four mature and healthy tree and shrub species were randomly chosen to sample their rhizosphere soils and first-order roots, i.e., the most distal and absorptive roots of the branching system (Ma et al. 2018), along these transects. The root samples of each species, which contained more than 50 first-order roots, were used to verify the mycorrhizal type and mycorrhizal colonization rates. We calculated the means for each species with 2 or more individuals to perform statistical analysis. All the selected species belonged to 28 genus and 19 families, with different life forms (tree and shrub) and leaf morphology (evergreen and deciduous) (Table S1).

Soil collection and analysis

Soils were sampled in late May 2018. The “root tracing from trunk” method and “soil adhering to roots after shaking” method (Chen et al. 2018; Phillips and Fahey 2006) were used to trace the first-order root and sample rhizosphere soil of specific plant species, respectively. Specifically, we identified a target species, dug (to 10 cm depth) along a main, coarse root that could be traced back to the trunk, and then picked out the branching fine roots (< 2 mm) and adhering soil. After shaking the fine roots gently, the soil adhering to the fine roots were carefully sampled with forceps and was defined as

rhizosphere soil. Bulk soil, i.e., soil not adhering to roots, from 0-10 cm depth was also sampled from four sampling sites at four slope positions at least 20 m apart along the altitudinal transect as a control. The fresh bulk and rhizosphere soil samples were sieved through a 2-mm mesh and stored at 4 °C until analysis. Soil and organic particles on the surface of the root samples were gently removed by forceps in deionized water (1 °C), and then instantly fixed in formalin-acetic acid-alcohol (FAA) solution at 4 °C until analysis (Li et al. 2017).

Mycorrhizal type and mycorrhizal colonization of plant species

A staining method followed by microscopic analysis (DM500, Frankfurt, Germany; 400 X magnification), modified from Li et al. (2017), were used to classify the mycorrhizal type of each species and the mycorrhizal colonization rates of the first-order roots according to root anatomical structures. Fifty first-order root segments were randomly selected to measure mycorrhizal colonization rates. Briefly, the root samples were washed in 10% (w/v) KOH solution at 90 °C for 30 min, acidified in 2% HCl at room temperature for 30 min, stained with 0.1% (w/v) acid fuchsin at 90 °C for 30 min and at 60 °C for 1 h, with a final wash in the solution of 1: 1: 1 lactic acid: glycerol: water. Roots were mounted in glycerin on microscope slides after being fully squashed. The appearance of coils (or arbuscules) in cortical cells indicated colonization by AM fungi, and the visual observation of the fungal sheath and Hartig net identified ECM fungi (Kong et al. 2014). The mycorrhizal colonization rates of each species was calculated as the number of roots colonized by mycorrhizal fungi divided by the total number of roots examined for a species (Kong et al. 2014; Kou et al. 2015).

Soil chemical variables

Soil pH was measured with a digital pH meter in a suspension of 1: 2.5 soil: water. Nitrate-N and ammonium-N were extracted with a 2 *M* KCl solution in a 1: 10 soil: solution ratio, shaken for 2 h and measured with a continuous flow analyzer (Bran Luebbe, AA3, Hamburger, Germany). Available-P was extracted with 0.5 *M* $NaHCO_3$ solution with a 1: 20 soil: solution ratio and shaken for 30 min. Soil total P (TP) was analyzed with a flow injection auto analyzer following digestion with H_2SO_4–$HClO_4$ digestion (Bao 2008). The content of soil organic C (SOC) was determined by the potassium dichromate volumetric method. The contents of total C (TC) and total N (TN) of dried and ground soils were determined by an elemental analyzer (Elementar, Vario Max CN, Frankfurt, Germany). The content of soil inorganic C (SIC) was calculated as the difference contents between TC and SOC.

Soil extracellular activities

Soil enzyme activities were measured following the methods of Saiya-Cork et al. (2002). Four enzyme activities, involved

in C- (β-1,4-glucosidase, βG), N- (β-1,4-N-acetylglucosaminidase, NAG and leucine aminopeptidase, LAP) and P- (phosphomonoesterase) acquisition, were assayed using fluorogenically-labeled substrates. These four soil enzymes are the most widely analyzed in the study of soil nutrient cycling. β-1,4-glucosidase hydrolyzes glucose from cellobiose. β-1,4-N-acetylglucosaminidase hydrolyzes glucosamine from chitobiose. Leucine aminopeptidase hydrolyzes leucine and other hydrophobic amino acids from the N terminus of polypeptides. Phosphomonoesterase hydrolyzes phosphate from phosphosaccarides and phospholipids (Sinsabaugh et al. 2009). We used 96-well microplates to perform enzyme assays. We used tris-aminomethane buffer with pH = 7 to make suspension for the determination of the enzyme activities. 7-amino-4-methyl coumarin was used to calibrate the activities of LAP, and 4-methylumbelliferone was used to calibrate the activities of the other enzyme activities. The fluorescence was measured using a microplate fluorometer (Synergy^{H4}, BioTek, USA) with excitation and emission filters of 365 and 450 nm, respectively.

Statistical analysis

The one-sample Kolmogorov-Smirnov test was used to test the normal distribution criteria of the data. Multi-way ANOVA was used to determine the differences in rhizosphere soil variables between different mycorrhizal type and life form and leaf morphology. Rhizosphere effects were calculated as: RE (%) = (R – B)/ B × 100%, R refers to the variables of rhizosphere soil, while B refers to the variables of bulk soil (Chen et al., 2018). Independent samples t-tests were used to compare the differences in variables between bulk soil and rhizosphere soil for each mycorrhizal plant. Linear regression analyses were used to investigate the relationships between mycorrhizal colonization rates, pH and C, N and P contents and C-, N- and P-acquisition enzyme activities. SPSS 17.0 software (Chicago, IL, USA) was used for the statistical analysis and Sigmaplot 10.0 software for the graphics.

Results

Mycorrhizal identification and colonization rates

Of the 34 woody species sampled, 25 AM and 9 ECM associations with plant species were observed (Table S1). Mycorrhizal colonization rates ranged from 17 to 98% for AM plants and 20 to 98% for ECM plants (Fig. 1a; Table S1).

Soil C, N and P contents in the rhizosphere soils

We just found mycorrhizal type and life form had interaction effects on the content of SIC in the rhizospheres ($p < 0.05$; Table S2). Mycorrhizal type alone affected soil N and P availabilities, while life form or leaf morphology did not (Table S2). The nitrate-N and available-P contents were 67% and 34% greater in the rhizosphere soil of AM plants compared to

those of ECM plants, respectively ($p < 0.05$; Fig. 1d and g). The pH in the rhizosphere soil was significantly different between each mycorrhizal plant type, with the most acidic values in the AM plants: the pH value was 0.49 units lower of AM plants compared to those of ECM plants ($p < 0.05$; Fig, 1i). No differences in rhizosphere SOC, TC, TN or TP contents were measured between different mycorrhizal plants ($p > 0.05$).

Ectomycorrhizal species had negative rhizosphere effect on nitrate-N content, while AM plants had positive rhizosphere effects on available-P content ($p < 0.05$; Fig. 1d and g). Arbuscular mycorrhizal plants had 26% smaller negative rhizosphere effect on the content of ammonium-N, when compared with ECM trees ($p < 0.05$; Fig. 1e). Arbuscular mycorrhizal plants increased soil acidity and had pH values 0.55 units lower in the rhizosphere than the bulk soil ($p < 0.05$; Fig. 1i). The pH in the rhizosphere soil of ECM plants were not significantly different to bulk soil ($p > 0.05$).

Soil C-, N- and P-acquisition enzyme activities in the rhizosphere soils

Mycorrhizal type and life form had interaction effects on βG activity in the rhizosphere soil ($p < 0.05$; Table S2). Mycorrhizal type alone affected soil N- and P-acquisition enzyme activities, while life form or leaf morphology did not (Table S2). The activities of LAP were 27% lower (Fig. 2b), while the phosphomonoesterase activities were 36% higher (Fig. 2c), respectively, in the rhizosphere soil of AM plants compared to ECM plants ($p < 0.05$). The ratio of C- to P-acquisition enzyme activity and the ratio of N- to P-acquisition activity in the rhizosphere soil of AM plants were 10% and 7%, respectively, lower than those under ECM associations ($p < 0.05$; Fig. 2e and f). This indicated relatively greater investment in P-acquisition enzymes in the rhizospheres of AM plants and relatively greater investment in C- and N-acquisition enzymes in the rhizospheres of ECM plants.

Relationships analyses

Linear regression analysis demonstrated that the content of nitrate-N was positively correlated with the activities of NAG (Fig. 3a; $p < 0.05$). The relative change in available P content was positively correlated with the activity of phosphomonoesterase, and was negatively correlated with soil pH ($p < 0.05$; Fig. 3c and d). The mycorrhizal colonization rate was positively correlated with the contents of nitrate-N and available-P and the activity of LAP in the rhizospheres of AM plants, and with the contents of SOC, TC and TN, as well as the activities of βG, NAG, LAP and phosphomonoesterase in the rhizospheres of ECM plants ($p < 0.05$; Fig. 4).

Discussion

Effects of mycorrhizal type on rhizosphere soil N and P availability

Ectomycorrhizal plants retained lower nitrate N and available P in the rhizosphere soil than AM plants in the subtropical karst forest (Fig. 1d and g). This is consistent with our first hypothesis and measurements made on mineral soils in temperate (Phillips et al. 2013) and tropical forests (Lin et al. 2018). The positive relationships between mycorrhizal colonization rates and nitrate-N and available-P contents in the rhizospheres of AM plants (Fig. 4a and b) suggests that AM colonization increased nutrient availability. Higher inorganic nutrient content in soils associated with AM plants is promoted by an integrated suite of traits that tend to improve the chemical quality of root and mycelial litter of AM plants, when compared with ECM plants (Jacobs et al. 2018; Kilpeläinen et al. 2019; Lin et al. 2018; Midgley et al. 2015), leading to faster litter decomposability and thus enhanced N and P mineralization rates (Lin et al. 2018; Phillips et al. 2013). Additionally, ectomycorrhizal fungi can produce extracellular enzymes accessing organic nutrient directly from SOM, while AM fungi cannot (Bödeker et al. 2016; Bunn et al. 2019). The direct organic N uptake implies the nutrient competition between ECM fungi and saprophytic microorganisms, which subsequently reduces SOM decomposition rates and soil N and P availabilities (Averill 2016).

Rhizosphere effects on soil nutrient contents have been used to assess the nutrient acquisition capacities of different plants (Lin et al. 2018). Arbuscular mycorrhizal plants caused positive rhizosphere effects on available-P content through an organic P 'mining strategy'. Rhizoexudate C released from AM plants stimulates rhizosphere soil microorganism to produce extracellular enzymes to promote P mineralization, which increase P availability (Keller et al. 2021; Kuzyakov and Blagodatskaya 2015; Yin et al. 2014), as evidenced by larger SOC contents and P-acquisition enzyme activity in the rhizosphere compared to bulk soil (Fig. 1b, 2c and 3c). Ectomycorrhizal trees, which caused stronger negative rhizosphere effects on mineralized N, may mainly reflect a greater 'scavenging strategy' than AM trees (Fig. S1). Given that TN content under ECM plants was similar to under AM plants (Fig. 1f), this result may suggest that ECM plants caused slower nutrient cycling rates, or they have greater N acquisition capacities that decrease mineral N contents in the rhizosphere soil, when compared with AM plants. Thus, AM plants appear important for mitigating N and P limitation than EM trees in karst forests. Ectomycorrhizal plants may also alleviate N limitation by increasing the secretion of large amount of N-acquisition enzymes (Cheeke et al. 2017). However, the greater acquisition capacities of ECM plants may reduce mineral N availability, due to rapid take-up of mineralized N and organic N (Zhang et al. 2019).

Effects of mycorrhizal type on rhizosphere soil potential enzyme activities

Partly consistent with our second hypothesis, ECM trees had higher N-acquisition enzyme activities in the rhizosphere soil, when compared with AM plants (Fig. 2b). This concurs with previously studies in temperate forests reported that potential enzyme activities that in the mineral soil were higher in ECM than AM plots (Cheeke et al. 2017; Phillips et al. 2013). Ectomycorrhizal fungi with larger saprophytic capability could produce larger amount of extracellular enzymes than AM fungi (Bödeker et al. 2016; Bunn et al. 2019). Soil enzyme activities were tightly coupled with mycorrhizal colonization rate of ECM fungi, but not AM fungi (Fig. 4), suggesting relative higher contribution of ECM fungi to soil extracellular enzyme activities than AM fungi. According to resource allocation theory, ECM plants and their rhizosphere soil microorganisms secrete more N-acquisition enzymes and increase the efficiency of N release to meet their greater N demands (Burns et al. 2013). The smaller mineralized N content under ECM plants suggested greater N absorptive capacity relative to AM plants reported herein supports this theory (Fig. 1d).

In contrast to N, AM plants retained higher P-acquisition enzyme activities in the rhizosphere soil, when compared with ECM plants (Fig. 2c), which was disagreed with the second hypothesis. Although AM fungi have relatively lower contribution to soil extracellular enzymes than ECM fungi (Fig. 4), as other study suggested (Cheeke et al. 2017; Phillips et al. 2013), they could enhance organic P acquisition by transferring C to promote the activity of P-solubilizing bacteria (Bunn et al. 2019; Zhang et al. 2018). Phosphorus-solubilizing bacteria that produce ALP, such as *Proteobacteria*, *Actinobacteria* and *Gemmatimonadetes*, play central roles in P-acquisition in karst soils (Hu et al. 2018). Thus, the P-acquisition enzymes in the rhizosphere soil of AM plants in this study may be derived from similar relationships with P solubilizing bacteria, but this was not confirmed. It is clear that high activities of P-acquisition enzymes under AM trees appear to have promoted organic P mineralization, as evidenced by strong relationship between phosphomonoesterase activity and available-P content (Fig. 3c). This suggested a stronger capacity of AM plant to adapt to low P condition by mineralizing organic P in this forest, when compared with ECM plants.

Enzyme stoichiometry clearly shifted to greater investment in N- rather than P-acquisition enzymes in the rhizosphere soil of ECM plants, relative to AM plants (Fig. 2f). This pattern was different from that observed in temperate forests where enzyme stoichiometry in ECM plots shifted to greater investment in both N- and P-acquisition enzymes relative to C-acquisition enzymes, compared to AM plots (Cheeke et al. 2017). The distinct pattern may reflect divergent plant-derived C use by different mycorrhizal types under different soil conditions. The fine roots of ECM trees released nearly three-fold more exudates to soil than the roots of AM trees in temperate forests (Yin et al. 2014). The large amount of root exudates released from ECM plants may stimulate associated fungi and saprophytic microorganisms to produce more extracellular

enzymes for both N and P acquisition (Cheeke et al. 2017; Phillips et al. 2013). However, AM and ECM plants might release similar C contents into rhizosphere soils in the present study (Fig. 1b and c). We speculated that ECM plants might allocate more C for producing N-acquisition enzymes to meet greater N demands, while AM plants may mainly utilize root-derived C for P-acquisition enzymes in the karst soil. However, more evidence is needed to support this hypothesis. The greater acquisition capacity of AM species for P and ECM species for N might suggest different niche of these contrast mycorrhizal plants, which helpful for the coexistence of these species. From the global scale, our results might provide an alternative explanation for the distributions of AM and ECM plants, i.e., AM plants dominate in low latitudes ecosystems with relatively P-limited soils, while ECM plants prevail in middle-high latitude soils with relatively poor N availability (Steidinger et al. 2019).

Effects of mycorrhizal type on rhizosphere soil pH

In partial support for our third hypothesis, the negative rhizosphere effect of AM plants on soil pH suggests that AM plant roots and/or the rhizosphere microbial community increased soil acidity (Fig. 1i). Although some studies demonstrated that ECM plants could secrete oxalate, while AM associations produce formate and acetate (Fransson et al. 2016; Toljander et al. 2007) to mobilize inorganic P from calcium phosphate minerals, the ability of ECM plants increased soil acidity was not obvious in this calcareous soils (Fig. 1i). Lower pH related to higher P availability in the rhizosphere of AM plants may suggest the important role of these plants in enhancing P acquisition, compared with ECM plants providing a competitive advantage to the former. However, ECM plants may grow more slowly or have evolved low P demand to adapt to P deficient soils. Chronic P limitation confers the primary stress on most trees and shrubs growing in karst soils (Du et al. 2010). Therefore, the greater ability of AM plants to alleviate P limitation may make these plants more competitive, compared to the stress-tolerator strategy of ECM plants, in the mixed forest.

Conclusion

Our results revealed that different nutrient economies were active in the rhizosphere soil of contrasting mycorrhizal plants in subtropical primary karst forest, and these were always enhanced compared to the adjacent bulk soil. We revealed how AM or ECM plants might affect rhizosphere soil N and P economies (Fig. 5). Ectomycorrhizal plants may invest more resource in the production of N-acquisition enzymes to adapt to lower N availability and meet greater N acquisition capacity (relative to AM plants), whereas AM associations may increase P availability (relative to bulk soils) by allocating more C to the production of P-acquisition enzymes that mineralize organic P, and by secreting acidic compounds that liberate P from calcium phosphate compounds. We suggest that these contrasting mycorrhizal associations have different N- and P-

acquisition capacity, which with implications for the coexistence of species and the maintenance of plant diversity in karst forests. However, it is not known whether the enzymes in the rhizosphere soils are secreted by plants or by microorganisms, or how non-mycorrhizal plants acquire nutrients (e.g. using proteoid roots) and contribute as a biodiverse mixed forest community as a whole for biogeochemical cycling in karst environments. Future work should include analysis of the origins of the enzymes to better reveal how individual plant-microbe and plant-plant interactions influence rhizosphere processes.

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Declarations

Conflicts of interest

The authors declare that they have no conflict of interest.

Availability of data and material

Requests for data or other materials should be directed to Xinyu Zhang (zhangxy@igsnrr.ac.cn).

Code availability

Not applicable.

Authors' contributions

X. Z. and X. W. planned and designed the research. X. Z., Y. Y., D. L. and Z. G. conducted fieldwork. Y. Y. performed experiments and analyzed data. Y. Y., X. Z., I. P. H., J. A. J. D. and T. A. Q. wrote the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

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451

Table and figure captions

Figure 1 Mycorrhizal colonization rates and C, N and P contents in the bulk and rhizosphere soil of different mycorrhizal plants

Values are expressed as means ± standard error. Different lowercase letters indicated the differences in rhizosphere soil between AM and ECM plants. * and ** indicated significant differences between bulk and rhizosphere soil at the level of $P < 0.05$ and $P < 0.01$, respectively. AM, plants associate with arbuscular mycorrhizae, n=25; ECM, plants associated with ectomycorrhizae, n=9; Bulk soil, n=4.

Figure 2 C-, N- and P-acquisition enzyme activities in the bulk and rhizosphere soil of different mycorrhizal plants

C_{en}, C- acquisition enzyme activity (the activity of β-1,4-glucosidase); N_{en}, N- acquisition enzyme activities (the activities of β-1,4-N-acetylglucosaminidase + leucine aminopeptidase); P_{en}, P-acquisition enzyme activities (the activities of phosphomonoesterase). Different lowercase letters indicated the differences in rhizosphere soil between AM and ECM plants. * and ** indicated significant differences between bulk and rhizosphere soil at the level of $P < 0.05$ and $P < 0.01$, respectively. AM, plants associate with arbuscular mycorrhizae, n=25; ECM, plants associated with ectomycorrhizae, n=9; Bulk soil, n=4.

Figure 3 Relationships between the availabilities of N and P, the activities of N- and P- acquisition enzyme activities and pH in the rhizosphere soil

Figure 4 Relationships between the mycorrhizal colonization rates and the contents of C, N and P and the activities of C-, N- and P- acquisition enzymes in the rhizosphere soil

Figure 5 A diagram of how AM and ECM plants affecting rhizosphere soil nutrient economies

Ectomycorrhizal associations may invest more C in the production of N-acquisition enzymes to adapt to lower N availability and greater N acquisition capacity (relative to AM plants) in the rhizospheres (a). Arbuscular mycorrhizal associations may increase rhizosphere soil P availability (relative to bulk soils) by allocating more C to produce P-acquisition enzymes that mineralizing organic P, and by secreting acidic compounds that liberate P from calcium compounds. The size of the arrows indicated the strength of the fluxes. Red arrow, C allocation; Orange arrow, effects on N availability; Blue arrow, effects on P availability; ↑, nutrient content increased in the rhizosphere soil relative to bulk soil; ↓, nutrient content decreased in the rhizosphere soil relative to bulk soil; --, nutrient content in the rhizosphere soil

was similar to that in the bulk soil.

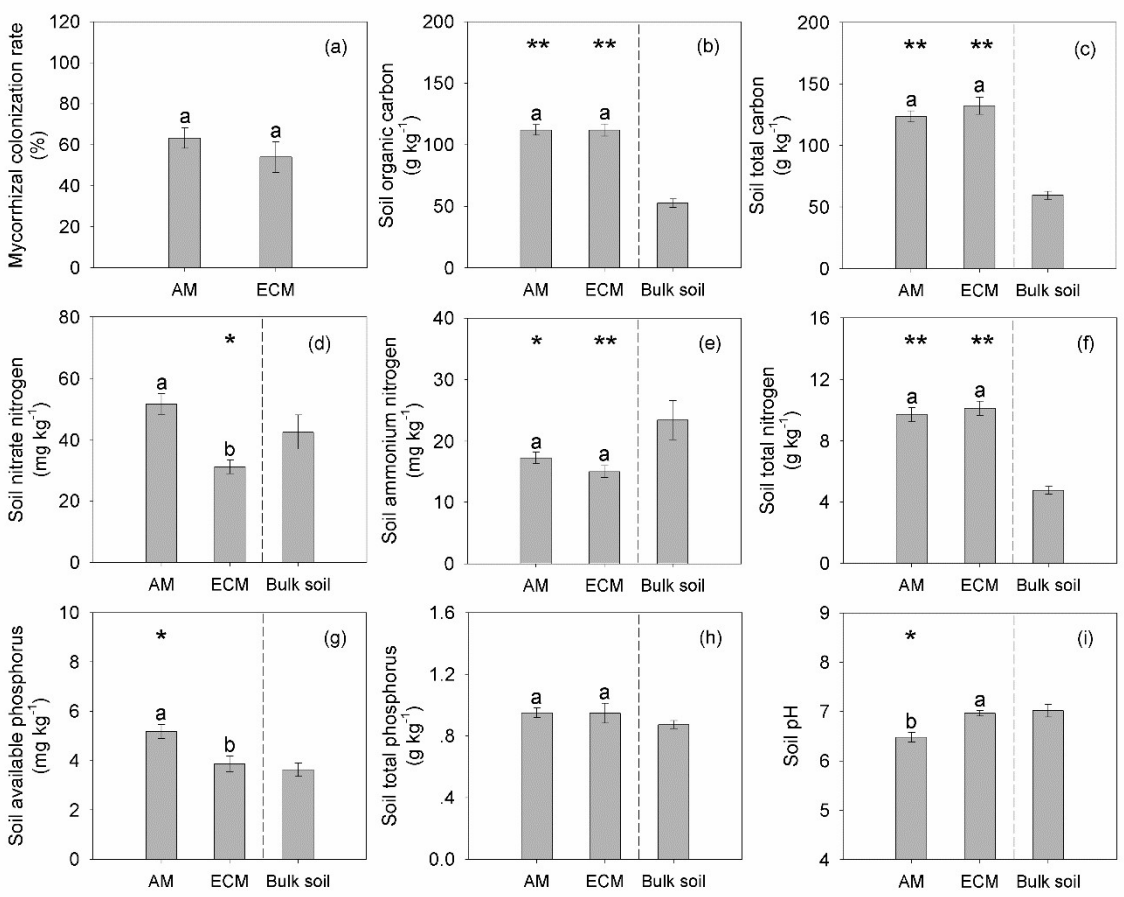
(a)
Mycorrhizal colonization rate (%)
AM
ECM
(b)
Soil organic carbon (g kg-1)
Bulk soil
(c)
Soil total carbon (g kg-1)
(d)
Soil nitrate nitrogen (mg kg-1)
(e)
Soil ammonium nitrogen (mg kg-1)
(f)
Soil total nitrogen (g kg-1)
(g)
Soil available phosphorus (mg kg-1)
(h)
Soil total phosphorus (g kg-1)
(i)
Soil pH

Fig. 1

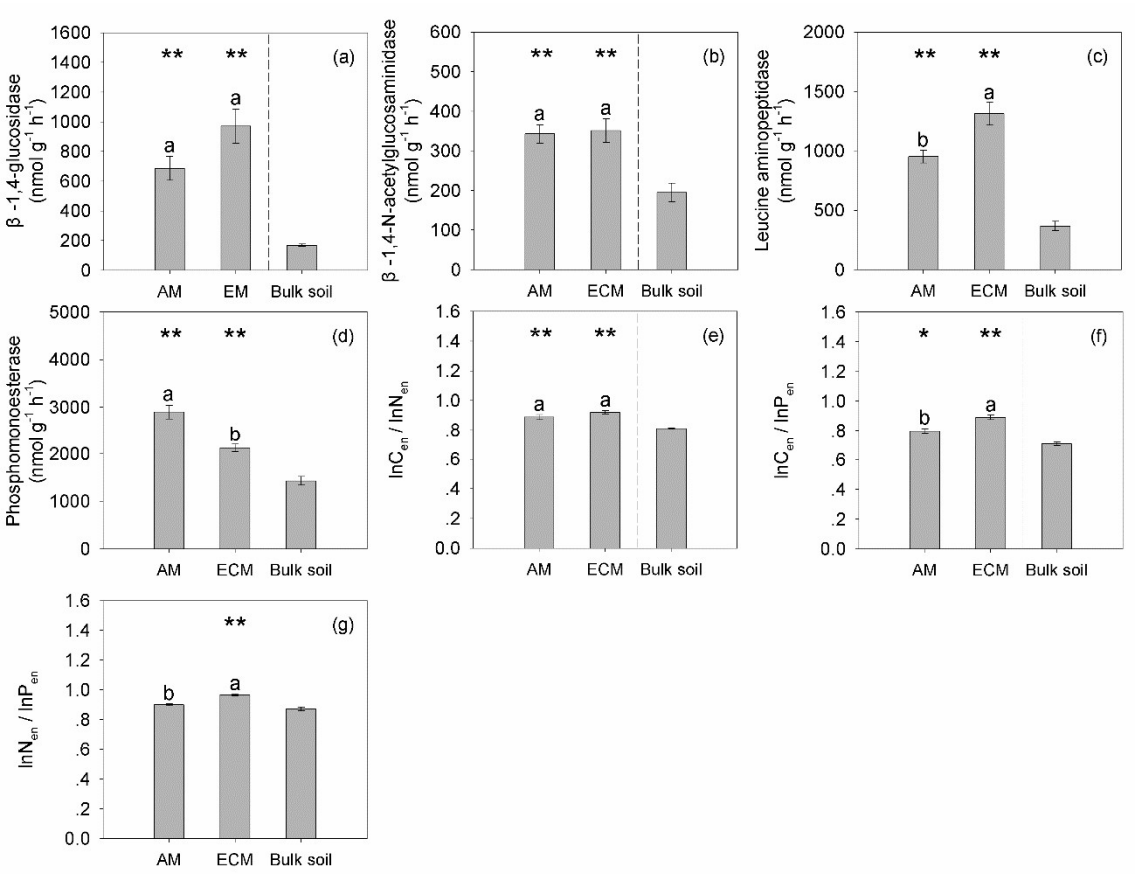
β -1,4-glucosidase (nmol g-1 h-1)
β -1,4-N-acetylglucosaminidase (nmol g-1 h-1)
Leucine aminopeptidase (nmol g-1 h-1)
Phosphomonoesterase (nmol g-1 h-1)
lnCen / lnNen
lnCen / lnPen
lnNen / lnPen
(a)
(b)
(c)
(d)
(e)
(f)
(g)
AM
EM
ECM
Bulk soil
**
*

Fig. 2

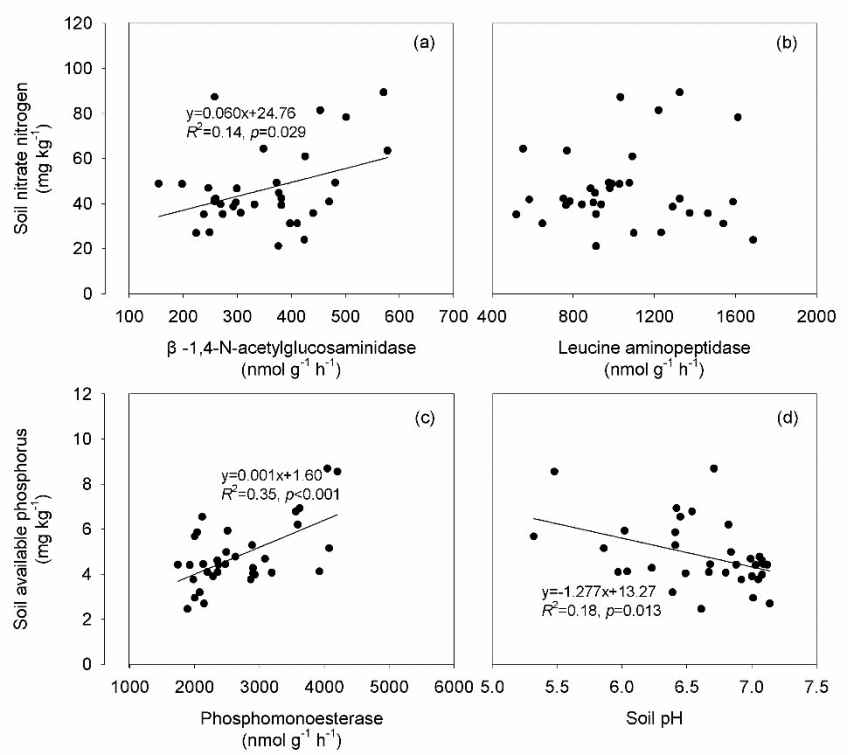
(a)
y=0.060x+24.76
R^2=0.14, p=0.029
Soil nitrate nitrogen (mg kg^{-1})
β -1,4-N-acetylglucosaminidase (nmol g^{-1} h^{-1})
(b)
Leucine aminopeptidase (nmol g^{-1} h^{-1})
(c)
y=0.001x+1.60
R^2=0.35, p<0.001
Soil available phosphorus (mg kg^{-1})
Phosphomonoesterase (nmol g^{-1} h^{-1})
(d)
y=-1.277x+13.27
R^2=0.18, p=0.013
Soil pH

Fig. 3

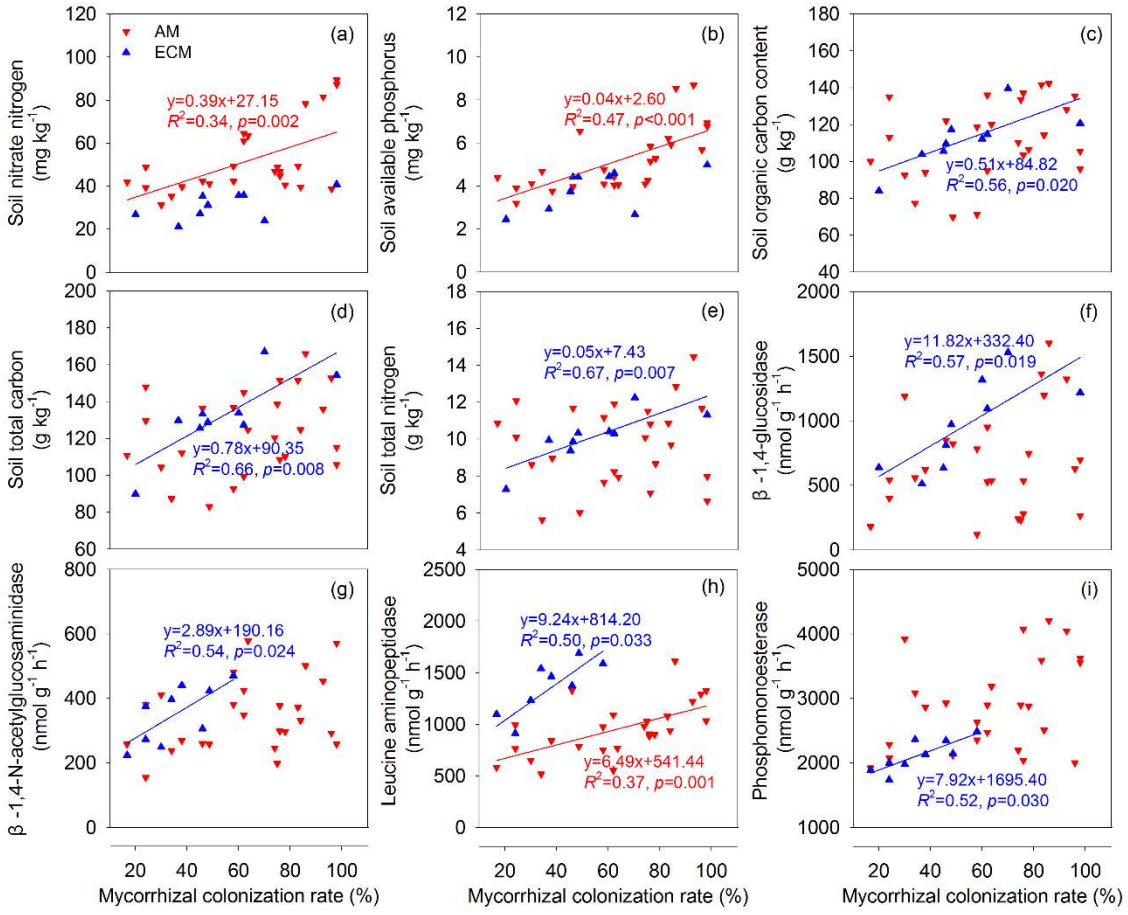
AM
ECM
(a)
y=0.39x+27.15
R2=0.34, p=0.002
Soil nitrate nitrogen (mg kg-1)
(b)
y=0.04x+2.60
R2=0.47, p<0.001
Soil available phosphorus (mg kg-1)
(c)
y=0.51x+84.82
R2=0.56, p=0.020
Soil organic carbon content (g kg-1)
(d)
y=0.78x+90.35
R2=0.66, p=0.008
Soil total carbon (g kg-1)
(e)
y=0.05x+7.43
R2=0.67, p=0.007
Soil total nitrogen (g kg-1)
(f)
y=11.82x+332.40
R2=0.57, p=0.019
β -1,4-glucosidase (nmol g-1 h-1)
(g)
y=2.89x+190.16
R2=0.54, p=0.024
β -1,4-N-acetylglucosaminidase (nmol g-1 h-1)
(h)
y=9.24x+814.20
R2=0.50, p=0.033
y=6.49x+541.44
R2=0.37, p=0.001
Leucine aminopeptidase (nmol g-1 h-1)
(i)
y=7.92x+1695.40
R2=0.52, p=0.030
Phosphomonoesterase (nmol g-1 h-1)
Mycorrhizal colonization rate (%)
Mycorrhizal colonization rate (%)
Mycorrhizal colonization rate (%)

Fig. 4

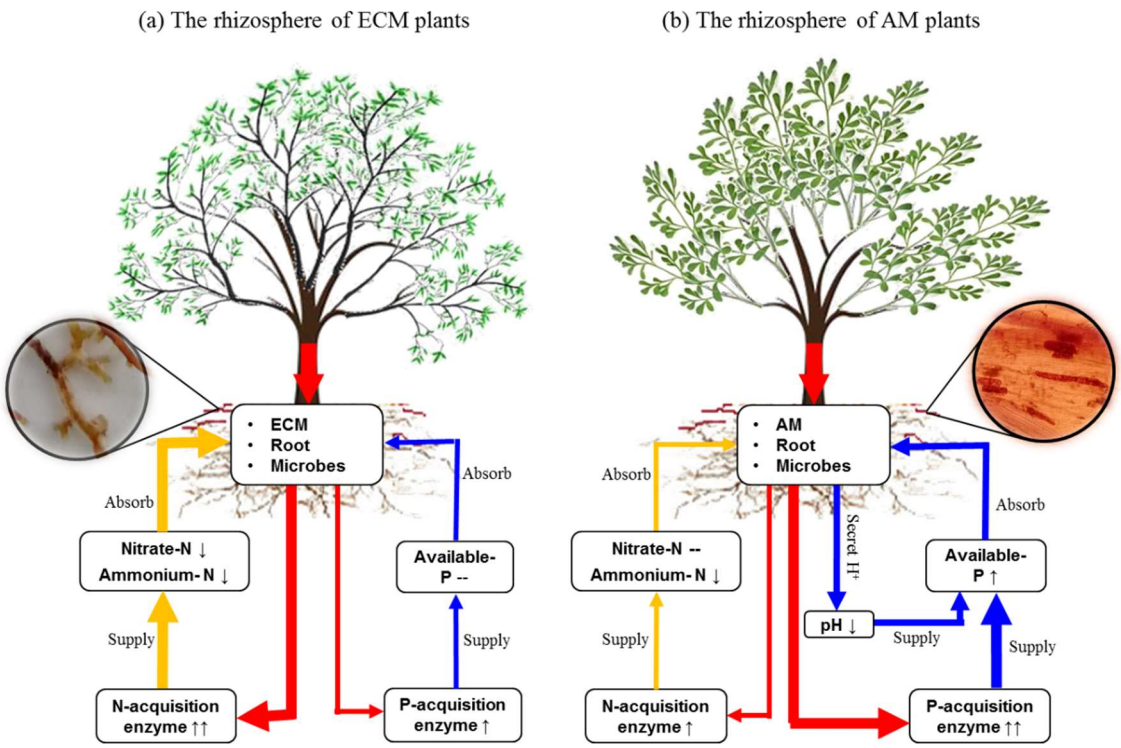
(a) The rhizosphere of ECM plants
(b) The rhizosphere of AM plants
ECM
Root
Microbes
Absorb
Absorb
Nitrate-N ↓
Ammonium- N ↓
Available-
P --
Supply
Supply
N-acquisition
enzyme ↑↑
P-acquisition
enzyme ↑
AM
Root
Microbes
Absorb
Absorb
Secret H+
Nitrate-N --
Ammonium- N ↓
Available-
P ↑
pH ↓
Supply
Supply
Supply
N-acquisition
enzyme ↑
P-acquisition
enzyme ↑↑

Fig. 5