Nutrient trade-offs mediated by ectomycorrhizal strategies in plants: Evidence from an Abies species in subalpine forests

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November 5, 2020

Abstract

1.Ectomycorrhizal (ECM) roots are evolutionary strategies of plants for effective nutrient uptake under varying abiotic conditions. Formation and morphological differentiations of ECM roots are important strategies in foraging environments. However, little is known on how such strategies mediate the nutrients of the below- and aboveground tissues and the balances among nutrient elements across environmental gradients. 2.We studied the function of ECM symbiosis in Abies faxoniana across its distributional range in Southwest China. The effects of differential ECM strategies, i.e. the contact exploration type, the short-distance exploration type, and the medium-distance exploration type, and root tips functional traits, etc., on root and foliar N and P and N:P ratio were examined across natural environmental gradients. 3.The ECM symbionts preferentially facilitated P uptake in A. faxoniana under both N and P limitations. The uptakes of N and P were primarily promoted by the effectiveness of ECM roots, e.g. ECM root tips per unit biomass, superficial area of ECM root tips, the ratio of living and dead root tips, but negatively related to the ECM proliferations and morphological differentiations. Generally, plant N and P nutrients were always promoted by the contact exploration type, while negatively affected by the short-distance exploration type in A. faxoniana. Root and foliar N and P nutrients were expected to be affected by the medium-distance exploration type in dynamics. Especially, root P limitation could be relieved when the frequency of medium-distance exploration type up to c.15%, whilst root N limitation was strengthen when the frequency of medium-distance exploration type over 20%. 4.We suggest that both below- and above-ground nutritional traits of host tree species could be strongly affected by ECM symbiosis in natural environments. The ECM strategies responding to environmental conditions significantly affect the plant nutrient uptakes and trade-offs. ECM soil exploration types are the great supplementary mechanisms for plant nutrient uptake.

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Keywords: *Abies faxoniana* ; ectomycorrhizal morphology; ectomycorrhizal strategy; plant N, plant P; plant N:P ratio; soil exploration types

1 INTRODUCTION

Over 80% of tree species in forest ecosystems could form ectomycorrhizal (ECM) symbionts. ECM fungi are irreplaceable to the health and growth of forest trees by enhancing soil nutrients uptake, particularly N and P elements (Smith & Read, 2008; Barrett *et al.*, 2011). It is certain that functional genes related to N and P exist in ECM fungi (Perez *et al.* , 2011; Cappellazzo *et al.* , 2008; Nehls *et al.* , 1999). ECM roots absorb and transport soil nutrients for host plants through root tips, being important adaptations to assistant to nutrient uptake (Figure 1), and emanating hyphae which are the important functional ECM traits. Varying emanates of ECM could be classified as different soil exploration types. Agerer (2001) defined the soil exploration types into five groups: contact exploration type (smooth mantle and only a few emanating hyphae), short-distance exploration type (ECM root with a voluminous envelope of emanating hyphae but no rhizomorphs), medium-distance exploration type (ECM root with rhizomorphs), long-distance exploration type (ECM root with long rhizomorphs), and pick-a-back exploration (ECM formed by members of the *Gomphidiaceae*). Different soil exploration types absorb soil water and nutrients at a different distance from the root tips (e.g. contact exploration with length of emanating elements 0 mm, short distance exploration type with length of emanating elements < 1 mm, and medium distance exploration type with length of emanating elements < 1 cm) (Tedersoo *et al.* , 2012; Pritsch & Garbaye, 2011); these features have important implications to plant nutrient absorption. The differentiation of ECM hyphae is an important feature of ECM, which could form different ECM morphologies (Agerer, 1987-2006; Agerer, 1991). ECM root tips, length of emanating hyphae or rhizomorphs, and morphological differentiation always responded to resources limitation, soil acidity, and climatic changes (Graefe *et al* ., 2010; Ostonen *et al* ., 2009; Toljander *et al* ., 2006; Rosinger *et al* ., 2018). Furthermore, changes of ECM root traits resulted in the variation of the nutrient uptake efficiency or ability in the host tree (Chen *et al* . 2016; Chen *et al* . 2018). The morphology, hyphae characteristics, and soil exploration types of ECMs are important foraging strategies for host plants to respond to environmental changes.

Nitrogen (N) and/or phosphorus (P) limitations are common in terrestrial ecosystems. Both N and P are important nutrient elements for plant growth and health. The ratio of N to P of plant tissue is generally used to indicate the nutrients limitation in plant growth and community health (Olde Venterink *et al.* , 2003; Güsewell & Koerselman, 2002). Generally, plant N:P ratio is a relatively stable trait. It was suggested that the values of N:P ratio < 14 or > 16, respectively, indicated N limitation or P limitation in plants (Güsewell & Koerselman, 2002; Tessier & Raynal, 2003). The value of plant N:P ratio is a helpful tool to diagnose the nutrient limitation condition or nutrient allocation partiality in individual (Koerselman & Meuleman, 1996). However, to some degree, P could be the ultimate limiting element in ecosystems, as evidenced by the fact that N nutrient uptake could be promoted when adding P (Vitousek *et al* ., 2010). N and P uptakes of tree species are strongly affected by climate, soil environmental conditions and nutrient acquisition capacity of the species (Güsewell, 2004; He *et al.* , 2008), as illustrated in Figure 1. Positive effects of rainfall but negative effects of air temperature on foliar N and P have been reported (Reich & Oleksyn, 2004; Han *et al.* , 2005). Climate eventually influences plant N:P ratios and productivity. He *et al* . (2008) believes that

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soil resources instead of climate play greater impacts on plant N and P, such that availability of soil N and P largely limits the nutrient uptake of plant species. Importantly, N and P nutrition in tree species largely depend on their nutrient capture ability of soil resources (Brandes*et al.* , 1998; Bardgett *et al.* , 2014).

The uptake efficiency of N and P is dependent of the root systems with strategies adapting to environmental conditions (Chien *et al.* , 2011; Hodge, 2004; Jackson & Caldwell, 1996). Of which, the ECM symbionts play an important role when tree species undergo environmental stresses (Alonso *et al.* , 2003; Ahonen-Jonnarth *et al.* , 2000) or nutrient deficiency (Almeida *et al.* , 2019; Hajong,*et al.* , 2013). The symbionts usually promote plant nutrients absorption by altering hyphae length, or modifying the morphology of root tips or microbial communities, etc., when plants are under stresses (Lõhmus *et al.* , 2006; Ostonen *et al.* , 2009; Boomsma & Vyn, 2008). Under natural environmental conditions, uptakes of N and P by roots are promoted by ECM foraging strategies, such as increases in ECM root tips and changes in hyphae length or morphology, etc. (Ostonen*et al.* , 2007; Ostonen *et al.* , 2011). The important foraging strategies discovered so far include the secretion of enzymes decomposing N or P complex by ECM root tips, and facilitation of nutrient acquisition far from the root distal by extending hyphae or rhizomorphs (Nehls & Plassard, 2018; Courty *et al.* , 2010; Pritsch & Garbaye, 2011). ECM plants are characteristically of low foliar nutrients and high leaf mass per unit area (especially*Pinaceae* and *Fagaceae*) (Koele *et al.* , 2012; Read, 1991; Cornelissenm *et al.,* 2001). The intimate connections of foliar N nutrition and ECM symbiosis are widely reported (Koele *et al* ., 2012; Hobbie & Hobbie, 2006; Hobbie *et al.* , 2005). For instance, isotope tracing experiments proved the transfers of N element among plant tissues and mycorrhizal fungi (Steven *et al* ., 2004; Hobbie & Högberg, 2012). Still, few studies reported the associations of ECM traits and foliar nutrients. There are observations of the associations of root and leaf nutrient traits (Craine & Lee, 2003; Tjoelker *et al.* , 2005) and reports of the positive correlations of N or P between roots and leaves (Liu *et al.* , 2010; Güsewell, 2004.). It is clearly known conceptually that mycorrhizal root systems assimilate N and P and then transfer them to shoots (Michelsen *et al.* , 1996; Smith & Read, 2008; Plassard & Dell, 2010) (Figure 1). Previous researches have revealed the relationship of foliar N with mycorrhizal fungi, asserting that mycorrhizal associations influence the foliar N transfer (Hobbie & Hobbie, 2006; Craine *et al* ., 2009). Controlled experiments demonstrated that the mycorrhizal symbionts affected the allocation of N and P nutrients among roots, stems, and leaves (Chen *et al.* , 2010; Johnson, 2010; Landis & Fraser, 2008; Wang *et al* ., 2006; Brandes *et al.* , 1998). Explicit knowledge concerning the distinctive impacts of ECM strategies on the root and leaf nutrients was required to be explored across the natural environment (Figure1). Combining the plant elemental stoichiometry with the ECM strategies can improve our understanding of the implications of ECM in the nutrition trade-offs, hence the health of plants. How ECM strategies mediate the below- and aboveground nutrients balance in plants in response to environmental changes is yet to be further explored.

Abies faxoniana is an ancient species in the genus *Abies*that experienced the glacial and interglacial periods (Florin, 1963). It is a typical ECM tree species and naturally distributed from 2700 to 3900 m *asl.* in subalpine area of Sichuan province, Southwest China. *A. faxoniana* forest is the primary vegetation type in that subalpine ecosystem. In this study, we investigated the effects of ECM strategies on the N and P nutrients between below- and aboveground tissues in plants under different environmental gradients. The root and foliar N and P contents, ECM traits representing nutrients uptake pathway (soil exploration types, ECM morphological diversity) and efficiency (fine root biomass, the ratio of living and dead root tips, the colonization ratio of the ECM root, ECM root tips per unit root biomass, the superficial area of ECM roots) were measured along the natural environmental gradients. Our objective was to determine how the ECM strategies in *A. faxoniana* regulated the nutrient preference of N and P nutrition and the nutrient uptake of the below- and aboveground. We hypothesized that (i) ECM strategies conduct the partiality of N and/or P nutrition of the below- and aboveground in *A. faxoniana* across the nature environmental gradients, and (ii) ECM soil exploration types distinctively regulate the nutrient uptakes in host tree.

2 MATERIALS AND METHODS

2.1 Study sites

A. faxoniana is mainly distributed in the western Sichuan province of China, from 30°N to 35°N in latitude.

Our study covered three sites along latitudinal gradients, including the Wolong Nature Reserve (latitude 30°53'N, longitude 102deg58'E), Miyaluo Nature Reserve (latitude 31deg42'N, longitude 102deg46'E), and Wanglang Nature Reserve (latitude 33deg00'N, longitude 104deg01'E).

Wolong Nature Reserve is located in the western Sichuan Plateau, with subtropical semi-humid climate, characterized by dry, cold winters and wet, cool summers (Li *et al.* , 2020); the mean annual temperature is 4.06 *C, and the mean annual precipitation is about 1062.8 mm. The dominant woody plant species consist of *A. faxoniana* , *Picea purpurea* Mast, *Betula albosinensis*Burkill, *Betula platyphylla* Suk, *Ribes tenue* Jancz,*Sorbus koehneana* Schneid, and *Rosa moyesii* , etc. The soil type is classified as dark brown soil in coniferous forest according to Chinese Soil Taxonomy (Zhang, 1983), which is developed from the weathering of slate of metamorphic rock (Taylor *et al.* , 2006).

Miyaluo Nature Reserve is also located in the western Sichuan Plateau, with subtropical semi-humid climate, characterized by dry, cold winters and wet, cool summers (Li *et al.* , 2013) and mean annual temperature of 1.67 *C and mean annual precipitation of 975.2 mm. The dominant tree species consists of *A. faxoniana* ,*Abies fabri* (Mast) Craib, *Picea purpurea* Mast, *Picea asperata* Mast, *Populus davidiana* Dode, and *Quercus aquifolioides* , etc. The main soil type is classified as dark brown soil in coniferous forest according to Chinese Soil Taxonomy, developed from the parental materials of phyllite, slate, and schist (Keyimu *et al.* , 2020).

Wanglang Nature Reserve is located in the Himalayas -Hengduanshan Mountains, with subtropical semi-humid climate, characterized by dry, cold winters and wet, cool summers (Zhao *et al.* , 2012) and mean annual temperature of 4.17 *C and mean annual precipitation of 1021.8 mm. The dominant tree species consists of*A. faxoniana, Picea purpurea* Mast*, Sabina saltuaria, Sabina squamata,* and *Betula albosinensis* Burkill, etc. The main soil type is classified as dark brown soil in coniferous forest according to Chinese Soil Taxonomy, with limestone as the main parental material (Taylor *et al.* , 2006).

2.2 Field sampling and processing

Root and soil samples were collected during June-August, 2018, at five elevations (i.e. 2850, 3000, 3194, 3413, and 3593 m *asl.*) in Wolong Nature Reserve, and two elevations in Miyaluo Nature Reserve (i.e. 3077 and 3612 m *asl.*) and Wanglang Nature Reserve (3070 and 3150 m *asl.*), respectively, using point-centered quarter sampling method (Mitchell, 2007) with randomly selected mature *A. faxoniana* trees ($n = 8$ focal trees per site) as center points. Four trees with DBH of 35-60 cm were sampled at each center point; these trees were all within 10 m distance from the focal tree. A 10 x 10 x 10 cm soil block was collected near the lateral root at 1 m away from each target tree after clearing the surface litter. Fine roots (diameter < 2 mm) were carefully separated from the soil, and then the soil samples from the same target tree were mixed to form a single composite sample, with a total of eight soil samples at each elevation on each site for chemical analysis. The field-collected root and soil samples were immediately placed in zip-lock plastic bags and stored in a cooler before being transported to laboratory for later processing. In laboratory, two random root samples for each center point were gently cleaned-washed with deionized water and stored in 5% glycerin at -20*C for ECM identification following the method of Kohle *et al.* (2018), and the other two samples were oven-dried at 48degC to constant weight and used for biomass measurement and chemical analysis. Fresh soil samples were frozen-stored at -20 degC until further processing and analysis.

We collected fully developed, current year leaves from each target tree evenly at northern, eastern, southern, and western directions and among the four target trees at each center point. The leaf samples for each center point were mixed and placed in envelope, and then stored in a cooler before being transported to laboratory for later processing. After clean-wash with deionized water, the foliar samples were oven-dried at 48degC until constant weight for chemical analysis.

2.3 ECM identification and classification

Root samples prepared in 5% glycerin were gently washed in running water and soil particles adhering to root tips were removed with fine forceps under a stereoscopic microscope. When roots were covered by fungal mantles, they were classified as ectomycorrhiza. The morphology of ECM was determined under

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a photographic stereo microscope (LEICA, M205FA, Germany), the macroscopic and microscopic characteristics of the mycorrhizae were identified based on Agerer (1987-2006) (i.e., ECM system, color, mantle surface structure, cystidia, emanating hyphae, rhizomorphs, etc.). The living and dead root tips were distinguished by discerning the freshness or elasticity of the root tips during the microscope observation, and the tip numbers of living and dead root tips in each soil block were counted and the ratio of living to dead root tips (Root-tips$_{ratio}$) were calculated. For representative ECM root samples of each morphotype in each soil block, three root tips were used for diameter (d , mm) and length (l , mm) measurements with the photographic stereo microscope. The total root tip number in each soil block was counted and identified by ECM morphotypes. The morphology diversity of ECM root tips (MDI) was measured by Simpson's index of diversity as in Lande (1996) and Matsuda & Hijii (2004); the ECM colonization ratio (C$_{ratio}$) was measured as the percentage of the infected root tips over the total root tips. The ECM root tips per unit root biomass (ECM$_{tips}$) were also measured. The superficial area of ECM root tips (SA) was measured for all ECM root tips in each soil block, with root tips determined as a combination of cylinder and hemisphere by:

SA (m^2 m^{-3}) $=\sum_{i=1}^{N}[(2\pi\,(\frac{di}{2})^2 + \pi\,\mathrm{d}_i\,(l_i - d_i\,))\mathrm{x}n_i\,]\mathrm{x}10^3$

where d_i represents the average diameter of the ECM root tips of morphotype i ; l_i represents the average length of the ECM root tips of morphotype i ; n_i represents the number of ECM root tips of morphotype i ; and N represents the total number of ECM morphology types.

We used the classification of Agerer (2001) and the Information System for Characterization and Determination of Ectomycorrhizae (DEEMY) database (*http://www.deemy.de/*) to assess the nutrient uptake strategies of ECM roots through exploration types. The ECM exploration types associated with *A. faxoniana* were categorized into contact (CE), short-distance (SDE) and medium-distance exploration (MDE) by the morphology types of ECM roots photographed with a stereo microscope. The CE type is described by the ECM roots with a smooth mantle and only a few emanating hyphae of negligible length, SDE by the ECM roots with a voluminous envelope of emanating hyphae of 0 – 1 mm in length but no rhizomorphs, and MDE by the ECM roots formed with rhizomorphs of 0.1 – 1 cm emanates. The frequency of ECM occurrence in each exploration type was calculated as the number of root tips of the specific type over the total root tip number.

2.4 Root and foliar chemical analysis

The oven-dried leaves and roots were analyzed for N and P concentrations on composite sampling by the central point. N concentration was measured by the elemental analyzer (vario EL III, CHNOS Elemental Analyzer, Elementar Analysensysteme GmbH, Germany). P concentration was measured by ICP (ICAP6300). The ratios of N to P in leaves and roots were calculated.

2.5 Soil chemistry

The soil samples were analyzed for pH, water content (SWC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), ammonium-nitrogen (NH$_4^+$-N), and nitrate-nitrogen (NO$_3^-$-N). Soil pH was measured with a pH meter (HI-9125, Hanna Instruments Inc, Woonsocket, RI) by mixing the air-dried soil sample with deionized water at a 1:2.5 ratio (w:v). SWC (%) was calculated from the mass loss after drying the fresh soil samples at 75°C to a constant weight, for at least 48 h. TN content was analyzed using the Kjeldahl digestion procedure (Gallaher *et al.* , 1976). TP was measured by ICP (ICAP6300). The AP, NH$_4^+$-N, and NO$_3^-$-N were determined by a continuous flow analyzer (SEAL AA3, Norderstedt, Germany). Soil organic carbon (SOC) content was measured by a K$_2$Cr$_2$O$_7$-H$_2$SO$_4$ calefaction method (Nelson & Sommers, 1982). Soil C:N ratio (C:N$_{soil}$) was calculated by SOC and TN. The measurements of TN, TP, and AP were all made on air-dried soil samples, the NH$_4^+$-N, and NO$_3^-$-N content were made on fresh soil samples.

The activities of acid phosphatase (ACP) and protease (PR) were measured on the frozen-stored soil samples. ACP was determined with *p* -nitrophenol as a substrate (Schinner *et al* ., 1996), with the reaction mixture of 1 g fresh soil with 1 ml 100 mM *p* -nitrophenol. PR was determined with casein as a substrate according to Ladd and Butler (1972), with the reaction mixture of 1 g fresh soil with 5 ml casein solution (2%, w/v).

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The enzyme activities were expressed as μmol g^{-1} soil h^{-1}.

2.6 Climatic data

The gridded daily dataset (CN05.1) with a spatial resolution of $0.25° \times 0.25°$ constructed by the "anomaly approach" during the interpolation with more 2400 station observations in China was employed to obtain the meteorological data for the study sites during 1997-2016 (New *et al.* , 2000; Xu *et al.* , 2009). In the "anomaly approach", we derived the final dataset by calculating a gridded climatology, and then adding a gridded daily anomaly to the climatology. The air temperature for each elevation on each site was derived through topographic correction with the lapse rate of air temperature set at $0.65°$C $(100$ m)$^{-1}$ (Zhao *et al.* , 2008). The same value of precipitation was assumed along the elevational gradient on each site. The temperature variables included the mean annual temperature (MAT), the mean temperature in the growing season (T_g), the annual mean maximum air temperature (T_{max}), and the annual mean maximum air temperature (T_{min}); the precipitation variables included the mean annual precipitation (MAP) and the mean precipitation in the growing season (P_g).

2.7 Statistical analyses

Descriptive statistical analysis of the changes in root and foliar concentrations of N and P and N:P ratios were performed by SPSS 17.0. Coefficient of variation (CV) across three study areas was calculated. For plant nutrient data were tested for normality of distribution using Lilliefors and Shapiro-Wilk tests, homogeneity of variance was tested using F and Levene tests. Multiple comparisons of plant nutrient variables mean were carried out using LSD's test for unequal sample sizes with 95% confidence intervals.

The "Varpart" function in the "Vegan" package was used to partition the variation of root and foliar nutrition traits (N and P contents, and N:P ratio, into components due to three categories of predictors (i.e. soil chemistry factors, climatic factors, and ECM trait factors) in RStudio.

By loading the "lavaan()" function in RStudio, we performed a structural equation models (SEMs) among soil factors, ECM traits and plant nutrients (N and P content, and N:P ratio). Firstly, we identified key soil factors and ECM traits by principal component analysis (PCA), and the first principal components and second principal components of soil factors (named Soil-PC1, Soil-PC2 respectively) and ECM traits (named ECM-PC1, ECM-PC2 respectively) were selected for SEMs analyses. Then we constructed SEMs for the effects of soil and ECM PC axes and climate factors (MAT, MAP) on root and foliar nutrients (i.e. concentrations of N and P and N:P ratio). Besides, Redundancy analysis (RDA) was used to determine the relationships of plant nutrient traits and ECM root traits across all sites and elevations. Hellinger or standardize transformation was used to transform the data of plant nutrient traits and ECM root traits for RDA.

Curve Estimation models were used to estimate the relationships of root and foliar nutrient variables with the colonization ratio of the soil exploration types ($n =9$). Of which, data of the colonization ratio of MDE and root P were transformed by SQRT for the regression analysis.

3 RESULTS

3.1 Variations in root and foliar N and P

The foliar N and P concentrations were the most variable, and root N and P concentrations were least variable, across the three study site and along the elevational gradients, as inferred by the CV values (Table 1). The mean root N concentration was significantly lower ($P< 0.05$) than the mean foliar N concentration, whilst the root N:P ratio was significantly higher ($P < 0.05$) than the foliar N:P ratio, across study areas and elevations.

Variance partitioning shows that the root N and P concentrations were mainly influenced by soil factors (Figure 2), with the root P concentration further affected by ECM traits with an explained variance of 10.8%. The variance in foliar N was mostly explained by the joint effect of the climate factors and the ECM traits. Among the different categories of factors, soil environmental conditions were most influential on the

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root and foliar N:P ratios, explaining 33.9% and 39.9% of the variations, respectively; whilst the ECM traits were secondary in affecting the root and foliar N:P ratios, explaining 14.8% and 9.18% of the variations, respectively.

3.2 Influences of ECM traits on root and foliar N and P

The results of PCA on soil variables show that PC1 and PC2 respectively explained 39.8% and 14.9% of the variations; the factors of high scores include ACP, pH, TN, NO_3^--N, TP, and SWC on PC1, and PR, $C:N_{soil}$, NH_4^+-N, and AP on PC2 (Table 2). For the ECM traits, PC1 and PC2 respectively explained 40.4% and 17.8% of the variations; factors of high scores include C_{ratio}, MDI, FRB, CE, SDE, SA, Root-tips$_{ratio}$, and ECM$_{tips}$ on PC1, and CE, MDE, and Root-tips$_{ratio}$ on PC2 (Table 2).

Figure 3 illustrates the direct or indirect effects of explanatory factors (soil variables, climatic factors, and ECM traits) on root and foliar nutrient traits in SEMs (Figure 3). The root P concentration was directly and significantly affected by MAT (δ = -0.48, SE = 0.12, $P < 0.001$), soil-PC1 (δ = -0.31, SE = 0.09, $P <$ 0.001), soil-PC2 (δ = -0.33, SE = 0.09, $P < 0.001$), and ECM-PC1 (δ = 0.25, SE = 0.11, $P < 0.05$), with an overall R^2 value of 0.55 (CFI = 0.99, SRMR = 0.03; Figure 3a1). The root N concentration was directly and significantly affected by soil-PC1 (δ = 0.31, SE = 0.09, $P < 0.001$), MAP (δ = -0.27, SE = 0.12, P < 0.05), MAT (δ = -0.56, SE = 0.12, $P < 0.001$), and ECM-PC2 (δ =0.15, SE=0.09, $P < 0.1$), with an overall R^2 value of 0.52 (CFI = 0.99, SRMR = 0.03; Figure 3a2). The root N:P ratio was directly and significantly affected by soil-PC1 (δ = 0.52, SE = 0.09, $P < 0.01$), soil-PC2 (δ = 0.18, SE = 0.09, $P < 0.05$), and ECM-PC2 (δ = 0.18, SE = 0.08, $P < 0.1$), with an overall R^2 value of 0.53 (CFI = 0.99, SRMR = 0.03; Figure 3a3).

The foliar P concentration was directly and significantly affected by soil-PC1 (δ = -0.21, SE = 0.11, $P <$ 0.1), soil-PC2 (δ =-0.24, SE = 0.11, $P < 0.05$), MAP (δ = 0.44, SE = 0.14, $P < 0.01$), MAT (δ = -0.30, SE = 0.15, $P < 0.05$), and ECM-PC1 (δ = -0.26, SE = 0.14, $P < 0.1$), with an overall R^2 value of 0.30 (CFI = 0.98, SRMR = 0.03; Figure 3b1). The foliar N concentration was directly and significantly affected by MAP (δ = 0.28, SE = 0.14, $P < 0.1$), MAT (δ = -0.41, SE = 0.15, $P < 0.01$), and ECM-PC2 (δ = -0.21, SE = 0.11, $P < 0.1$), with an overall R^2 value of 0.25 (CFI = 0.98, SRMR = 0.03; Figure 3b2). The foliar N:P ratio was directly and significantly affected by soil-PC1 (δ = 0.44, SE = 0.11, $P < 0.01$), soil-PC2 (δ = 0.45, SE = 0.11, $P < 0.01$), MAP (δ = -0.41, SE = 0.13, $P < 0.01$), and ECM-PC1 (δ = 0.25, SE = 0.13, $P <$ 0.1), with an overall R^2 value of 0.37 (CFI=0.98, SRMR=0.03; Figure 3b3).

The soil and climatic factors also imposed indirect effects on root and foliar nutrients by influencing ECM traits (Figure 3). While the ECM-PC1 significantly affected root and foliar P concentrations and foliar N:P ratio, it was significantly affected by soil-PC1 (δ = -0.28, SE = 0.09, $P < 0.01$), soil-PC2 (δ = -0.29, SE = 0.01, $P < 0.01$), MAT (δ = -0.36, SE = 0.11, $P < 0.01$), and MAP (δ = 0.34, SE = 0.11, $P < 0.01$). MAT had a significant effect on ECM-PC2 (δ = -0.40, SE =0.12, $P < 0.05$), which significantly affected root and foliar N concentrations and root N:P ratio.

The RDA axis 1 (RDA1) and axis 2 (RDA2) respectively explained 38.7% and 2.4% of the variations in root nutrient variables, and 25.5% and 1.23% of the variations in foliar nutrient variables (Figure 4). In roots, there were significant positive relationships of P concentration with C_{ratio} and Root-tips$_{ratio}$, between N concentration and SA, and of the N:P ratio with FRB and MDI; both root N and P concentrations were negatively related to the SDE, MDI, and FRB (Figure 4a). In leaves, there were significant positive relationships between P concentration and CE, of N concentration with C_{ratio}, Root-tips$_{ratio}$, SA, and CE, and of N:P ratio with FRB and MDI, respectively. While foliar N concentration was negatively related to SDE, MDI, and FRB, the foliar P was negatively to SDE, MDE MDI, and FRB (Figure 4b).

3.3 Relationships of root and foliar nutrients with soil exploration types

Root P concentration was positively related to the colonization ratio of CE with an exponential relationship (R^2=0.73, $P < 0.01$; Figure 5a). Root P and N concentrations were negatively related to the colonization ratio of SDE with a linear (R^2=0.57, $P < 0.05$; Figure 5b) and exponential relationships (R^2=0.65 $P < 0.001$;

Figure 5c), respectively. As shown in Figure 5d, it suggested that root P varied with the colonization ratio of MDE in a curve relationship, and increased firstly with the increase of the colonization ratio of MDE, then decreased with the increase of MDE. Root N:P ratio decreased with the increase of the colonization ratio of MDE (R^2=0.52, $P < 0.05$; Figure 5e). As to foliar nutrient variables, foliar N:P was decreased with the colonization ratio of MDE in less than 20%, yet increased with that of MDE when over 20% (R^2=0.56, $P < 0.09$; Figure 5f).

4 DISCUSSION

It is generally uncontroversial that ECM fungi promote the uptake of N and P in plants. While the root N and P nutrition of ECM plants were widely investigated (Franklin *et al* ., 2014; Almeida *et al* ., 2019; Zhang *et al* ., 2019), relatively few studies have attempted to determine the attributes of ECM symbionts to aboveground nutrition in tree species (Michelsen *et al.* , 1996; Koele *et al.* , 2012). In this study, we examined the effects of the variations in ECM symbiosis on root and foliar N and P nutrition in *A. faxoniana* across certain natural environmental gradients. Generally, the ECM in *A. faxoniana* played more an important role on P uptake than N uptake under both N and P limitations (Figure 2, Figure 3, Figure 6). We found that the root N and P concentrations in *A. faxoniana* were more strongly related to the ECM traits than the foliar N and P concentrations (Figure 3), and the ECM soil exploration types played different impacts on root and foliar N concentrations or N:P ratio (Figure 5). During the process, soil resources and climatic factors appeared as the primary drivers of the ECM strategies. Our findings allow us to develop a conceptual model on the intervention of ECM symbiosis on root and foliar N and P nutrition using *A. faxoniana* as a case study (Figure 6). The model illustrates that the ECM strategies strongly affect the root nutrients, and then through the interconnections between roots and aboveground tissues in nutrient transportation and re-allocations, eventually influence the foliar nutrients, with preferential effects on P under both N and P limitations.

4.1 Differential effects of ectomycorrhizal strategies on the below- and aboveground plant N and P nutrients

Concerning our first hypothesis, we confirmed the distinctive effects of ECM strategies on plant N and P elemental stoichiometry in roots and leaves across nature environmental gradients. In this study, the mature *A. faxoniana* trees were deficient in root P (Table 1, P concentration: 0.72±0.13 mg g^{-1}) as well as both root and foliar N (values of N concentration <10 mg g^{-1} and N:P ratio <14) as judged by the normal standards (Güsewell & Koerselman, 2002; Güsewell, 2004; Tessier & Raynal, 2003). P is generally more limiting than N in terrestrial ecosystems as it is derived primarily from rock weathering and uniquely depended on root systems (Walker & Syers, 1976; Vitousek *et al.* , 2010). According to the results in this study, the variations of ECM traits in *A. faxoniana* contributed more to root and foliar P concentrations than N concentrations (Figure 2; Figure 3), suggesting that ECM strategies are more functional on P uptakes than on N uptakes under both N and P limitations. Basically, the resource allocation in belowground by the mycorrhizal symbiosis is expected to abide by the nutrition requires of plant species (Merrild *et al.* , 2013). However, the priority in nutrient acquisition is frequently determined by the strategic choices of plant species under several nutrient element limitations. It has been recognized that the ECM symbiosis give priority to the uptake of P but not N when in deficient supplies under different experimental conditions (Smith *et al* ., 2011; Zavišić *et al* ., 2016; Almeida *et al.* , 2019). Moreover, it has been reported that the ECM symbioses sometimes do not largely alleviate N limitation (Näsholm *et al.* , 2013; Franklin *et al* ., 2014), and that plants could obtain N by the root pathway rather than the mycorrhizal symbioses which would require extra C investment under N shortage (Jiang *et al.* , 2017; Zhang *et al.* , 2019).

The way of nutrient acquisition might change the nutrition preferences in plant species (Houlton *et al.* , 2007; Zhang *et al.* , 2018). Apart from soil resources and climatic factors, our study shows that the varied ECM traits greatly influenced N and P nutrients in *A. faxoniana* (Figure 2, Figure 3). Overall, the ECM traits implying the uptake efficiency, such as the colonization ratio of ECM root tips, the ratio of the living to dead root tips, the colonization ratio of the contact exploration type, and the superficial area of ECM root tips, were all positively related to the below- and aboveground N and P concentrations in *A. faxoniana* (Figure

4). However, fine root biomass and morphological diversity of ECM roots performed negative influence on plant N and P concentration but positive influence on root and foliar N:P ratio, suggesting that the trade-offs of *A. faxoniana* between the improvement of ECM root proliferation and morphology differentiation requiring more C invested and the nutrients uptake. Accordingly, we could conclude that both N and P nutrients of roots and leaves in *A. faxoniana* are primarily facilitated by the nutrient uptake efficiency of ECM roots, while the N and P stoichiometry is strongly related to the alteration of uptake or transportation pathway of ECM roots. Researches show that the nutrient uptake efficiency of the symbiotic fungi in plants might mediate the concentration of the nutrients in roots and leaves, e.g. the ECM colonization ratio, ECM absorption root vigor (Vandenkoornhuyse *et al* ., 2003; Beltrano *et al.* , 2013; Li *et al.* , 2015), ECM root tips biomass per stand basal area, and absorptive capacity of ECM emanates (Ostonen *et al.* , 2011), etc.; whilst the N and P stoichiometry in plant species could be affected by the function of mycorrhizal symbionts, e.g. hyphae exploration ability and/or extracellular enzyme secretion (Chen *et al.* , 2010), etc. Plant nutrients uptake and balance exceedingly depends on the alternative foraging strategies of the ECM root systems (e.g. foraging precision of hyphae, morphology plasticity, and foraging scale) under different environmental conditions (Wang *et al* ., 2006; Köhler *et al.* , 2018; Einsmann *et al.* , 1999; Chen *et al.* , 2018). While controlled experiments and isotope tracing studies have demonstrated that ECM symbionts contribute to the improvements of plant biomass, foliar N and P acquisition (Brandes et al., 1998; Hobbie & Hobbie, 2006; Craine et al., 2009), such functional roles are not readily observable in natural ecosystems due to the confounding effects of biotic and biotic environments. In this study, under the natural environmental gradients, we were able to reveal the differential roles of ECM strategies in N and P uptake in *A. faxoniana* .

4.2 Trade-offs of nutrient uptake and soil exploration types in *Abies faxoniana*

As the different impacts of soil exploration types on root and foliar N and P nutrients, we confirmed the hypothesis about the differences in nutrient uptake of soil explorations types. We found that the concentrations of root and foliar N and P in *A. faxoniana* were positively associated with the frequency of contact exploration type (Figure 3; Figure 4a, b; Figure 5a), and negatively with that of the short-distance and the medium-distance exploration types (Figure 4a, b). Especially, root N and P decreased with the improvement of the frequency of short-distance exploration type, while concentration of root P varied with the frequency of the medium-distance exploration type in 9%-30% with a quadratic relationship in this study (Figure 5b, c, d). This suggests that the extension of emanates across natural environmental gradients may simply be a passive response of ECM fungi to N and P deficiency rather than for facilitating N and P uptakes of host trees. This is different with these findings which often demonstrate positive relationships between the nutrient status of host plants and the length of emanates of ECM roots (Agerer, 2001; Brandes *et al* ., 1998; Hobbie & Agerer, 2010; Lilleskov *et al.* , 2011). In this study, the soil N and P were mostly deficient across the study sites (alkaline N: 40.78±18.83 mg g^{-1}, total P: 0.87±0.26 mg g^{-1}). It is likely that the contact exploration type and the short-distance exploration type facilitated the uptake of alkaline N and available P, whereas the medium-distance exploration type helped forage the organic N and P far from the root distal (Agerer, 2001; Hobbie & Agerer, 2010). Our study showed the facilitations of the contact exploration type in nutrient uptake (Figure 4; Figure 5). Interestingly, we found that root P limitation could be relieved when colonization ratio of medium-distance exploration type up to *c.* 15%, whilst root N limitation was strengthening when the frequency of medium-distance exploration type over 20% (Figure 5e). It suggested the converse or negative ecological function on plant nutrient uptake among the soil exploration types which need considerable C requirement despite the host tree under both heavy nutrient limitations. Ostonen *et al.* (2007, 2011) proposed that host tree always relied on the high efficiency of resource capture of the root-mycorrhiza continuum whilst investing little C to ECM root systems. Besides, plants would cut down the investment when C allocation outweigh the benefit obtained from ECM fungi (Johnson *et al.* 2003; Treseder, 2004), or ECM fungi sometimes hold the nutrients for itself in priority resulting in host tree remaining nutrient deficient under extreme nutrient limitations (Treseder & Allen, 2002). The improved root and foliar N and P by the occurrence of the contact exploration type and the negative relationships with the frequency of the short-distance and the medium-distance exploration types may partially attribute to trade-offs between the

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C allocation to ECM emanates and nutrient uptake in host plants (Johnson *et al.* , 2013; Magyar *et al.* , 2007).

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FIGURE CAPTIONS

FIGURE 1 Conceptual model of the role of ECM symbionts in plant N and P uptake

FIGURE 2 Schematic diagram of variation partitioning in determining the effects of soil variables, climate factors, and ECM traits on root and foliar nutrient variables. Values in diagram represents the explained variations in each category of factors and various interactions.

FIGURE 3 Analysis of structural equation models (SEMs) on root and foliar nutrient variables. *, $P < 0.1$; **, $P<0.05$; ***, $P<0.01$. Standardized path coefficients (δ) were showed on line arrows. R^2 value represents the proportion of total variance explained for the specific dependent variable. (a1): SEMs depicting the regulatory pathway of the controls in root P; (a2): SEMs depicting the regulatory pathway of the controls in root N; (a3): SEMs depicting the regulatory pathway of the controls in root N:P; (b1): SEMs depicting the regulatory pathway of the controls in leaf P; (b2): SEMs depicting the regulatory pathway of the controls in leaf N; (b3)**:** SEMs depicting the regulatory pathway of the controls in leaf N:P. ECM-PC1: the first principal components of ECM traits by PCA; ECM-PC2: the second principal components of ECM traits by PCA. MAT: the mean annual temperature; MAP: the mean annual precipitation. Soi-PC1: the first principal components of soil factors principal component analysis (PCA); Soi-PC2: the second principal components of soil factors by PCA.

FIGURE 4 Redundancy analysis (RDA) ordination biplot of ECM traits and root nutrients (a) and foliar nutrients (b). C_{ratio}: Colonization ratio of ECM fungi; CE: ECM roots with contact exploration type; ECM_{tips}: ECM root tips per unit root biomass; FRB: Fine root biomass; MDE: ECM roots with medium exploration type; MDI: Morphology diversity index; Root-tips$_{ratio}$: the ratio of the living to dead root tips; SA: superficial area of ECM; SDE: ECM roots with short distance exploration type; MiNR1: sampling at 3077 m *asl* . in Miyaluo Nature Reserve; MiNR2: sampling at 3612 m *asl* . in Miyaluo Nature Reserve; WaNR1: sampling at 3070 m *asl* . in Wanglang Nature Reserve; WaNR2: sampling at 3150 m *asl* . in Wanglang Nature Reserve; WoNR1: sampling at 2850 m *asl* . in Wolong Nature Reserve; WoNR2: sampling at 3000 m *asl* . in Wolong Nature Reserve; WoNR3: sampling at 3194 m *asl* . in Wolong Nature Reserve; WoNR4: sampling at 3413 m *asl* . in Wolong Nature Reserve; WoNR5: sampling at 3593 m *asl* . in Wolong Nature Reserve.

FIGURE 5 The significant relationships of root and foliar nutrients with soil exploration types. (a): the regressions of root P and the colonization ratio of CE; (b): the regressions of root P and the colonization ratio of SDE; (c): the regressions of root N and the colonization ratio of SDE; (d): the regressions of sqrt(root P) and sqrt(the colonization ratio of MDE); (e): the regressions of root N:P and the colonization ratio of MDE; (f): the regressions of foliar N:P and the colonization ratio of MDE. CE: ECM roots with contact exploration type; SDE: ECM roots with short-distance; MDE: ECM roots with medium exploration type.

FIGURE 6 A conceptual model of the intervention of ECM symbiosis on root and foliar N and P nutrients in *A. faxoniana* . I: The primary effects of ECM symbiosis on root nutrients. Root N and P nutrients could both be strongly affected by ECM symbiosis, but the effects were stronger on root P than root N; II: Indirect mediation of ECM symbiosis on foliar N and P nutrients driven by the nutrient limitation signals from leaves to roots; III: Changes in foliar N and P nutrients caused by variations in ECM strategies. Changes in ECM foraging strategies imposed greater influences on foliar P than foliar

(**a**)

(**b**)

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(a)

R^2=0.73 P < 0.01
y=0.52exp(1.05x)

Root P (mg g^{-1})

Colonization ratio of CE (%)

(b)

R^2=0.65 P < 0.001
y=0.85-1.137x

Root P (mg g^{-1})

Colonization ratio of SDE (%)

(c)

R^2=0.57 P < 0.05
y=15exp(-1.856x)

Root N (mg g^{-1})

Colonization ratio of MDE (%)

(d)

R^2= 0.54 P = 0.09
y=-0.489+6.533x-7.752x^2

Sqrt(root P)

Sqrt(colonization ratio of MDE)

(e)

R^2=0.52   P < 0.05
y=9.43+(0.977/x)

Root N:P

Colonization ratio of MDE (%)

(f)

R^2= 0.54 P < 0.09
y=27.37-266.38x+1134.46x^2-1502.66x^3

Foliar N:P

Colonization ratio of MDE (%)