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

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Abstract

1. Ectomycorrhizal (ECM) symbiosis is an evolutionary biological trait of higher plants for effective nutrient uptakes. However, little is known that how the formation and morphological differentiations of ECM roots mediate the nutrients of below- and aboveground plant tissues and the balance among nutrient elements across environmental gradients. Here, we investigated the effects of ECM foraging strategies on root and foliar N and P concentrations and N:P ratio *Abies faxoniana* under variations of climate and soil conditions.

2. The ECM symbionts preferentially mediated P uptake under both N and P limitations. The uptake efficiency of N and P was primarily associated with the ECM root traits, for example, ECM root tip density, superficial area of ECM root tips, and the ratio of living to dead root tips, and was affected by the ECM proliferations and morphological differentiations. The tissue N and P concentrations were positively associated with the abundance of the contact exploration type and negatively with that of the short-distance exploration type.

3. Our findings indicate that the nutritional status of both below- and aboveground plant tissues can be strongly affected by ECM symbiosis in natural environments. Variations in the ECM strategies in response to varying environmental conditions significantly influence plant nutrient uptakes and trade-offs.

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 form ectomycorrhizal (ECM) symbionts, which are essential for maintenance of forest ecosystem health and effective soil nutrient uptakes by host trees (Barrett et al., 2011; Smith & Read, 2008). The ECM roots facilitate soil nutrient uptake through branching root tips and emanating hyphae. The ECM-infested roots vary greatly in shape and structural

configurations (Agerer, 1987–2006; Agerer, 1991) and are functionally differentiated in the capacity of soil exploration range. Agerer (2001) classified the ECM roots into five types based on the extent of soil exploration: the contact exploration (smooth mantle and only a few emanating hyphae), the short-distance exploration (ECM root with a voluminous envelope of emanating hyphae but no rhizomorphs), the medium-distance exploration (ECM root with rhizomorphs), the long-distance exploration (ECM root with long

rhizomorphs), and the pick-a-back exploration (ECM formed by members of the *Gomphidiaceae*). The five ECM exploration types differ in their capability of reaching out for soil resources at distances from the root tips through variations in the length of emanates (Pritsch & Garbaye, 2011; Tedersoo et al., 2012). The types and morphologies of ECM are found to respond to variations in soil and climatic conditions (Graefe et al., 2010; Ostonen et al., 2009; Rosinger et al., 2018; Toljander et al., 2006), and greatly affect the nutrient uptake capacity and efficiency of the host trees (Chen et al., 2016, 2018).

Both N and P are essential nutrient elements for plants and other organisms, but their limitations are common in terrestrial ecosystems. The uptake efficiency of N and P is dependent on 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 trees undergo environmental stresses (Ahonen-Jonnarth et al., 2000; Alonso et al., 2003) or nutrient deficiency (Almeida et al., 2019; Hajong et al., 2013). The symbionts help improve soil nutrient absorption by altering hyphae length, modifying the morphologies of root tips, or affecting microbial communities, when trees are under stresses (Boomsma & Vyn, 2008; Lõhmus et al., 2006; Ostonen et al., 2009). Under natural environmental conditions, uptakes of N and P by roots are enhanced by ECM foraging strategies in favor of extended exploration of soil resources (Ostonen et al.,2007, 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 (Courty et al., 2010; Nehls & Plassard, 2018; Pritsch & Garbaye, 2011).

ECM plants are characteristically of low foliar nutrients and high leaf mass per unit area, especially the tree species in Pinaceae and Fagaceae families (Cornelissen et al., 2001; Koele et al., 2012; Read, 1991). The intimate connections of foliar N nutrition and ECM symbiosis are widely reported (Hobbie et al., 2005; Hobbie & Hobbie, 2006; Koele et al., 2012). For instance, isotope tracing experiments provided direct evidence of the N transfers among plant tissues and mycorrhizal fungi (Hobbie & Högberg, 2012; Steven et al., 2004). Still, few studies have reported the associations between 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 (Güsewell, 2004; Liu et al., 2010). The mycorrhizal root systems are known to have the capability of assimilating N and P and then transferring them to shoots (Michelsen et al., 1996; Plassard & Dell, 2010; Smith & Read, 2008). Previous research has revealed the relationship of foliar N with mycorrhizal fungi, asserting that mycorrhizal associations influence the foliar N transfer (Craine et al., 2009; Hobbie & Hobbie, 2006). Controlled experiments demonstrated that the mycorrhizal symbionts affected the allocation of N and P nutrients among roots, stems, and leaves (Brandes et al., 1998; Chen et al., 2010; Johnson, 2010; Landis & Fraser, 2008; Wang et al., 2006). However, how ECM strategies

mediate the below- and aboveground nutrients balance in plants in response to environmental changes yet remains unelucidated.

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 2,700 to 3,900 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 nutrient uptake between below- and aboveground tissues in plants under different environmental gradients, that is, varying mean annual temperatures, mean annual rainfall, elevations, and soil types. The root and foliar N and P contents, ECM traits representing nutrient uptake pathway, and efficiency were measured. Our objective was to determine how the ECM strategies in *A. faxoniana* regulated the nutrient preference of N and P nutrition in below- and aboveground tissues. We hypothesized that (a) ECM strategies mediate the partiality of N and P nutrition in below- and aboveground tissues in *A. faxoniana* in response to environmental variations, and (b) ECM soil exploration types differentially regulate the nutrient uptakes in host trees.

2 | MATERIALS AND METHODS

2.1 | Study sites

Abies faxoniana is exclusively distributed in the western Sichuan Province of China, from 30°N to 35°N in latitude. Our study covered three sites along the latitudinal gradient, including the Wolong Nature Reserve (latitude 30°53′N, longitude 102°58′E), Miyaluo Nature Reserve (latitude 31°42′N, longitude 102°46′E), and Wanglang Nature Reserve (latitude 33°00′N, longitude 104°01′E).

Wolong Nature Reserve is located in the western Sichuan Plateau, with subtropical semihumid climate, and 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 1,062.8 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, *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 semihumid climate, and characterized by dry, cold winters and wet, cool summers (Li et al., 2013). The mean annual temperature is 1.67°C, and the mean annual precipitation is 975.2 mm. The dominant tree species consists of *A. faxoniana*, *Abies fabri* (Mast) Craib, *Picea purpurea* Mast, *Picea asperata* Mast, *Populus davidiana* Dode, *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 semihumid climate, and characterized by dry, cold winters and wet, cool summers (Zhao et al., 2012). The mean annual temperature is 4.17°C, and the mean annual precipitation is 1,021.8 mm. The dominant tree species consists of *A. faxoniana*, *Picea purpurea* Mast, *Sabina saltuaria*, *Sabina squamata*, *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 (2,850, 3,000, 3,194, 3,413, and 3,593 m *asl.*) in Wolong Nature Reserve, and two elevations in Miyaluo Nature Reserve (3,077 and 3,612 m *asl.*) and Wanglang Nature Reserve (3,070 and 3,150 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 diameter at breast height (DBH) of 35–60 cm were sampled at each center point; these trees were all within 10 m distance from the focal tree. A $10 \times 10 \times 10$ 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 Köhle et al., (2018), and the other two samples were oven-dried at 48°C to constant weight and used for biomass measurement and chemical analysis. Fresh soil samples were frozen-stored at −20°C 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 48°C until constant weight for chemical analysis.

2.3 | ECM identification and classification

Root samples prepared in 5% glycerin were gently washed in running tap 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 a photographic stereo microscope (Leica, M205FA, Germany), and 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, and rhizomorphs). 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$_{ratio}$) was 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 and 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 samples in each soil block, with root tips determined as a combination of cylinder and hemisphere by:

$$SA\,(m^2 m^{-3}) = \sum_{(i=1)}^{N} \left[\left(2\pi \left(\frac{d_i}{2} \right)^2 + \pi d_i (l_i - d_i) \right) \times n_i \right] \times 10^3$$

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 exploration (CE), short-distance exploration (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 | Chemical analyses

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.

The soil samples were analyzed for pH, water content (SWC), total N (TN), total P (TP), available N (AP), ammonium N (NH_4^+-N), and nitrate N (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 hr. 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 C (SOC) content was measured by a $K_2Cr_2O_7$-H_2SO_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, and the NH_4^+-N and NO_3^--N contents 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 in 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 in 5 ml casein solution (2%, w/v). The enzyme activities were expressed as μmol/g soil h^{-1}.

2.5 | Climatic data

The gridded daily climate dataset (CN05.1), with a spatial resolution of 0.25° × 0.25° constructed by the "anomaly approach" during the interpolation with more 2,400 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, 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).

2.6 | Statistical analyses

Descriptive statistical analysis of the changes in root and foliar concentrations of N and P and N:P ratios was performed by SPSS 17.0. Coefficient of variation (CV) across three study sites was calculated. Plant nutrient data were tested for normality of distribution using the Lilliefors and Shapiro–Wilk tests, and the homogeneity of variance was tested using F and Levene's tests. Multiple comparisons of plant nutrient variables 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 under 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 variables, ECM traits and plant nutrients (N and P contents, and N:P ratio). Firstly, we identified key soil variables and ECM traits by principal component analysis (PCA), and the first and second principal components of soil variables (named Soil-PC1 and Soil-PC2, respectively) and ECM traits (named ECM-PC1 and 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). Redundancy analysis (RDA) was used to determine the relationships of plant nutrient traits and ECM root traits across all sites and elevations. Hellinger or standardized 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 most variable, and root N and P concentrations were least variable, across the three study sites and along the elevational gradient, as inferred by the CV values (Table 1). The mean root N concentration was significantly lower

TABLE 1 Descriptive statistics of root and foliar N and P nutrients in *Abies faxoniana* across the three study sites

Statistical parameter	Foliar N:P	Root N:P	Foliar N (mg/g)	Foliar P (mg/g)	Root N (mg/g)	Root P (mg/g)
N	72	72	72	72	72	72
Mean	8.47b	15.40a	17.44a	2.24c	10.81b	0.72c
SD	1.86	3.85	6.98	1.22	2.06	0.13
CV	21.97%	25.01%	40.02%	54.76%	19.05%	17.43%

Note: Different letters designate significant difference among nutrient variables at 0.05 level.

(p < .05) than the mean foliar N concentration, while the root N:P ratio was significantly higher (p < .05) than the foliar N:P ratio, across sites and elevations.

Variance partitioning shows that the root N and P concentrations were predominantly influenced by soil factors (Figure 1), 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 root and foliar N:P ratios, explaining
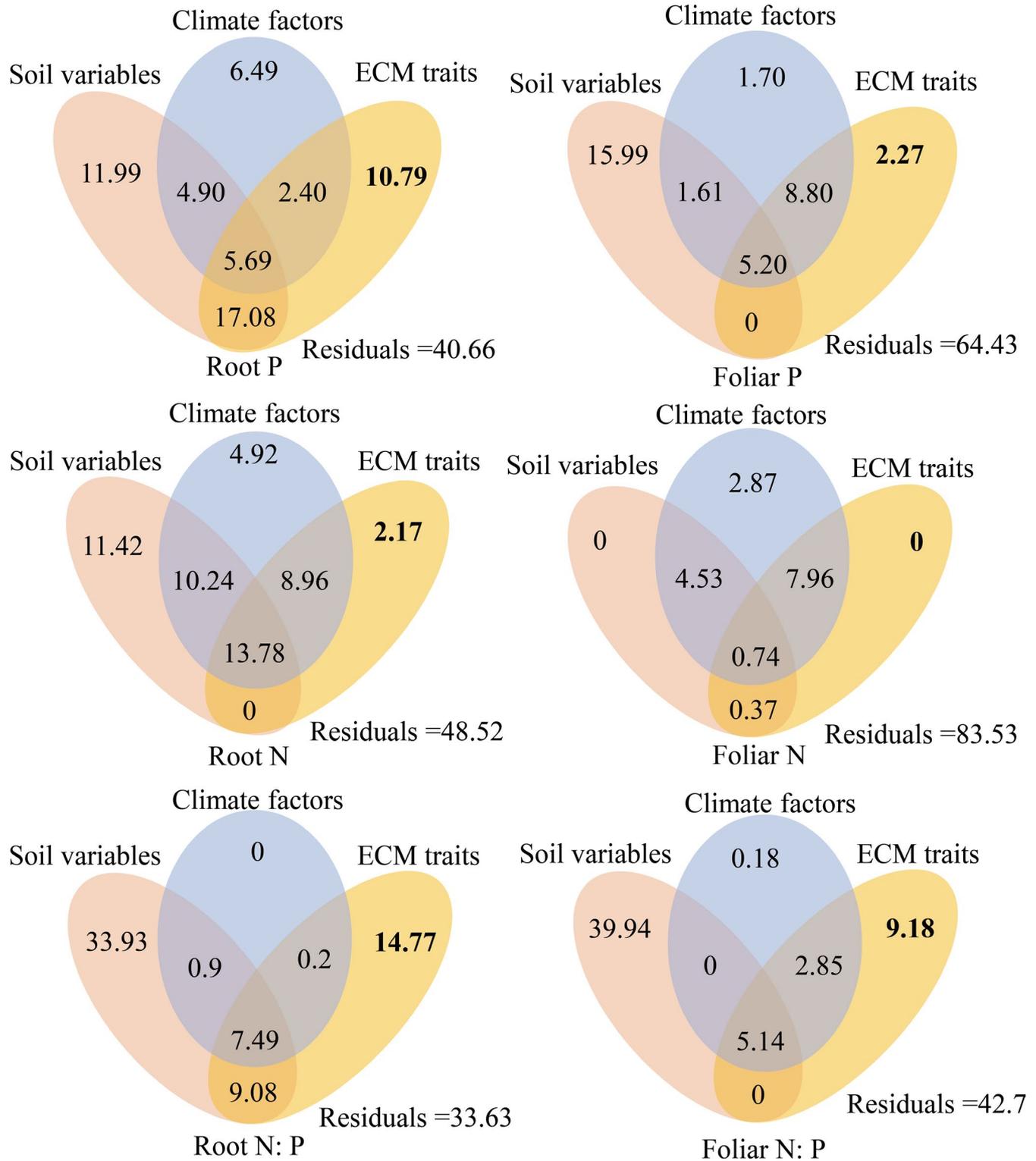
FIGURE 1 Schematic diagram of variation partitioning in determining the effects of soil variables, climate factors, and ECM traits on root and foliar nutrient variables. Values in diagram represent the explained variations in each category of factors and various interactions

33.9% and 39.9% of the variations, respectively, while 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 variables with 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; variables with 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).

The SEMs illustrate that the ECM traits affected root and foliar nutrient variables, in addition to the direct and significant effects of soil variables and climate factors (Figure 2). Root nutrients were positively affected the ECM traits, while the foliar nutrients, with exception of foliar N:P, were negatively affected. The SEMs explained 55% of the variance in root N concentration, 52% in root P concentration, and 53% in root N:P ratio, respectively. ECM-PC1 had a significant and positive correlation ($p < .05$) with root P concentration, and a marginal correlation ($p < .1$) with root N concentration and N:P ratio (Figure 2a1–3). The variances explainable by SEMs in foliar N concentration, P concentration, and N:P ratio were at 30, 25, and 37%, respectively. Both foliar P concentration and the N:P ratio were marginally correlated ($p < .1$) with ECM-PC1, while foliar N concentration was marginally correlated ($p < .1$) with ECM-PC2 (Figure 2b1–3).

The soil variables and climate factors also imposed indirect effects on root and foliar nutrients by influencing ECM traits. While root and foliar P concentrations and foliar N:P ratio were significantly affected by ECM-PC1, this component was significantly affected by soil-PC1, soil-PC2, MAT, and MAP (Figure 2). MAT had a significant effect on ECM-PC2, which in turn 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 nutrients, and 25.5% and 1.23% of the variations in foliar nutrients (Figure 3). There were significant positive relationships of root P concentration with C$_{ratio}$ and Root-tips$_{ratio}$, between root N concentration and SA, and of the root N:P ratio with FRB and MDI; both root N and P concentrations were negatively correlated to the SDE, MDI, and FRB (Figure 3a), whereas 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 the N:P ratio with FRB and MDI, respectively. The foliar N concentration was negatively correlated to SDE, MDI, and FRB, and the foliar P was negatively correlated to SDE, MDE MDI, and FRB (Figure 3b).

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 ($R^2 = 0.73$, $p < .01$; Figure 4a). Root P and N concentrations were negatively related to the colonization ratio of SDE with a linear ($R^2 = 0.57$, $p < .05$; Figure 4b) and exponential relationships ($R^2 = 0.65$ $p < .001$; Figure 4c), respectively. As illustrated in Figure 4d, root P concentration varied with the colonization ratio of MDE in a curvilinear relationship, and increased firstly with the increase in the colonization ratio of MDE, then decreased with the increase in MDE. Root N:P ratio decreased

TABLE 2 Scores of factors on PC1 and PC2 in the soil variables and ECM traits based on principal component analysis (PCA)

	Factors	PC1	PC2		Traits	PC1	PC2
Soil variables	SWC	1.52	−0.19	ECM traits	FRB	−0.78	−0.09
	pH	0.89	0.43		Root-tips$_{ratio}$	1.31	−0.73
	TN	1.49	−0.15		C$_{ratio}$	1.4	−0.55
	C:N$_{soil}$	0.13	−0.76		ECM$_{tips}$	0.88	0.43
	NH_4^+-N	0.62	−1.09		CE	1.24	0.89
	NO_3^--N	1.22	0.4		SDE	−0.8	−0.43
	PR	0.56	0.76		MDE	0.22	−1.49
	TP	0.88	0.06		MDI	−1.4	−0.37
	AP	−0.12	−1.09		SA	0.97	−0.37
	ACP	1.51	−0.14				

Abbreviations: NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; ACP, soil acid phosphatase; AP, available phosphorus; C:N$_{soil}$, Soil C:N ratio; CE, ECM roots of contact exploration type; C$_{ratio}$, Colonization ratio of ECM fungi; ECM$_{tips}$, ECM root tips per unit root biomass; FRB, fine root biomass; MDE, ECM roots of medium-distance exploration type; MDI, morphology diversity index; PR, soil protease; Root-tips$_{ratio}$, the ratio of the living to dead root tips; SA, superficial area of ECM; SDE, ECM roots of short-distance exploration type; SWC, soil water content; TN, total nitrogen; TP, total phosphorus.
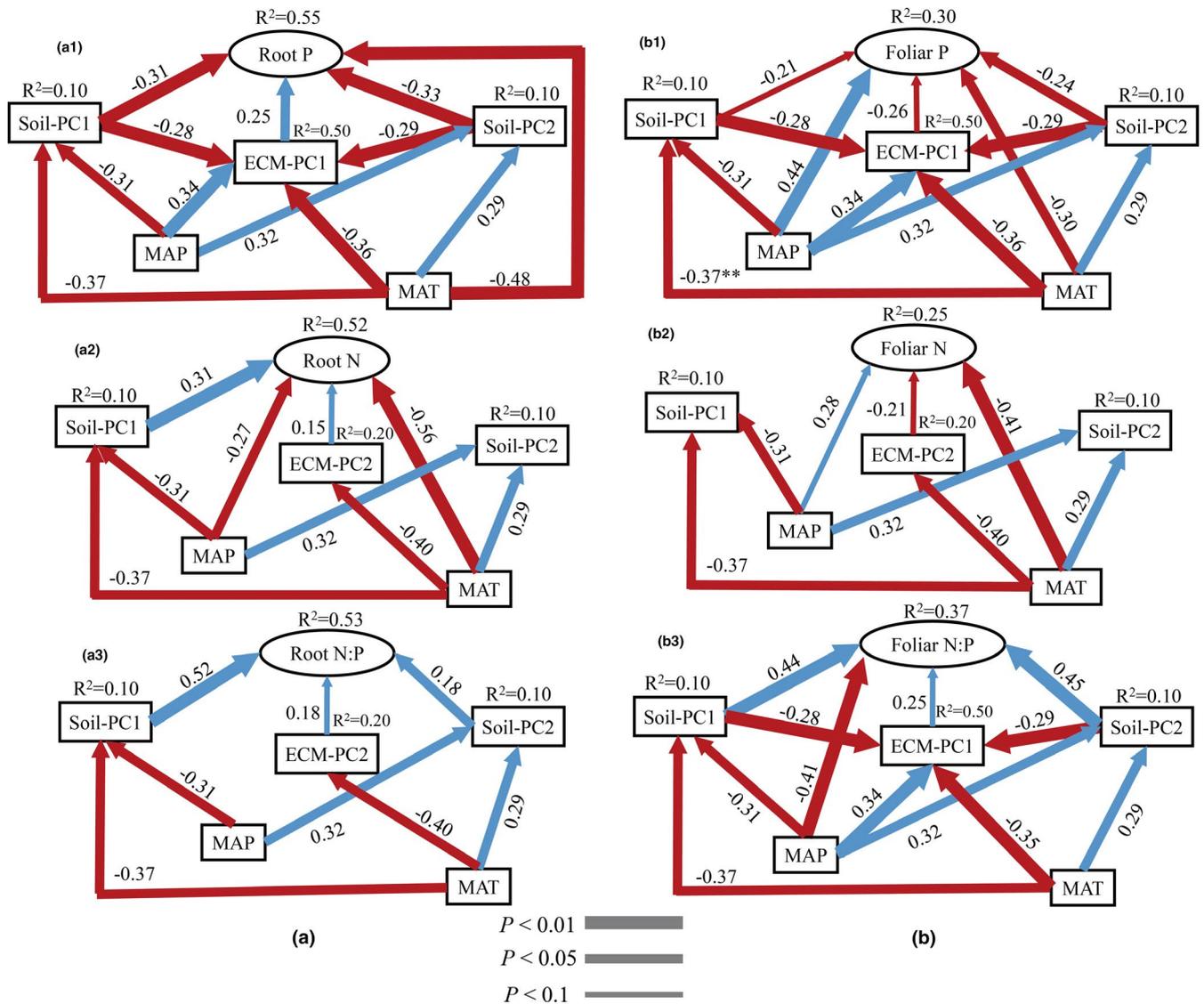
FIGURE 2 Analysis of structural equation models (SEMs) on root and foliar nutrient variables. Standardized path coefficients (δ) are displayed on line arrows; significant paths are color-coded in blue if positive, and in red, if negative. R^2 value represents the proportion of total variance explained for the specific dependent variable. (a1): SEMs depicting the regulatory pathway of the controls on root P; (a2): SEMs depicting the regulatory pathway of the controls on root N; (a3): SEMs depicting the regulatory pathway of the controls on root N:P; (b1): SEMs depicting the regulatory pathway of the controls on foliar P; (b2): SEMs depicting the regulatory pathway of the controls on foliar N; (b3): SEMs depicting the regulatory pathway of the controls on foliar 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

with the increase in the colonization ratio of MDE ($R^2 = 0.52$, $p < .05$; Figure 4e). The foliar N:P ratio decreased with the colonization ratio of MDE at values < 20%, increased at values > 20% ($R^2 = 0.56$, $p < .09$; Figure 4f).

4 | DISCUSSION

It is widely recognized that ECM fungi promote the uptake of N and P in plants. While the root N and P nutrition of ECM plants has been widely

studied (Almeida et al., 2019; Franklin et al., 2014; Zhang et al., 2019), relatively few studies have attempted to determine the attributions of ECM symbionts to aboveground nutrition in tree species (Koele et al., 2012; Michelsen et al., 1996). In this study, we examined the effects of the variations in ECM symbiosis on root and foliar N and P nutrition in *A. faxoniana* under varying soil types and climate factors. Generally, the ECM in *A. faxoniana* appeared to be more important in P uptake than N uptake under both N and P limitations (Figures 1, 2, and 5). The ECM traits in *A. faxoniana* were better correlated with root N and P concentrations than with the foliar N and P concentrations
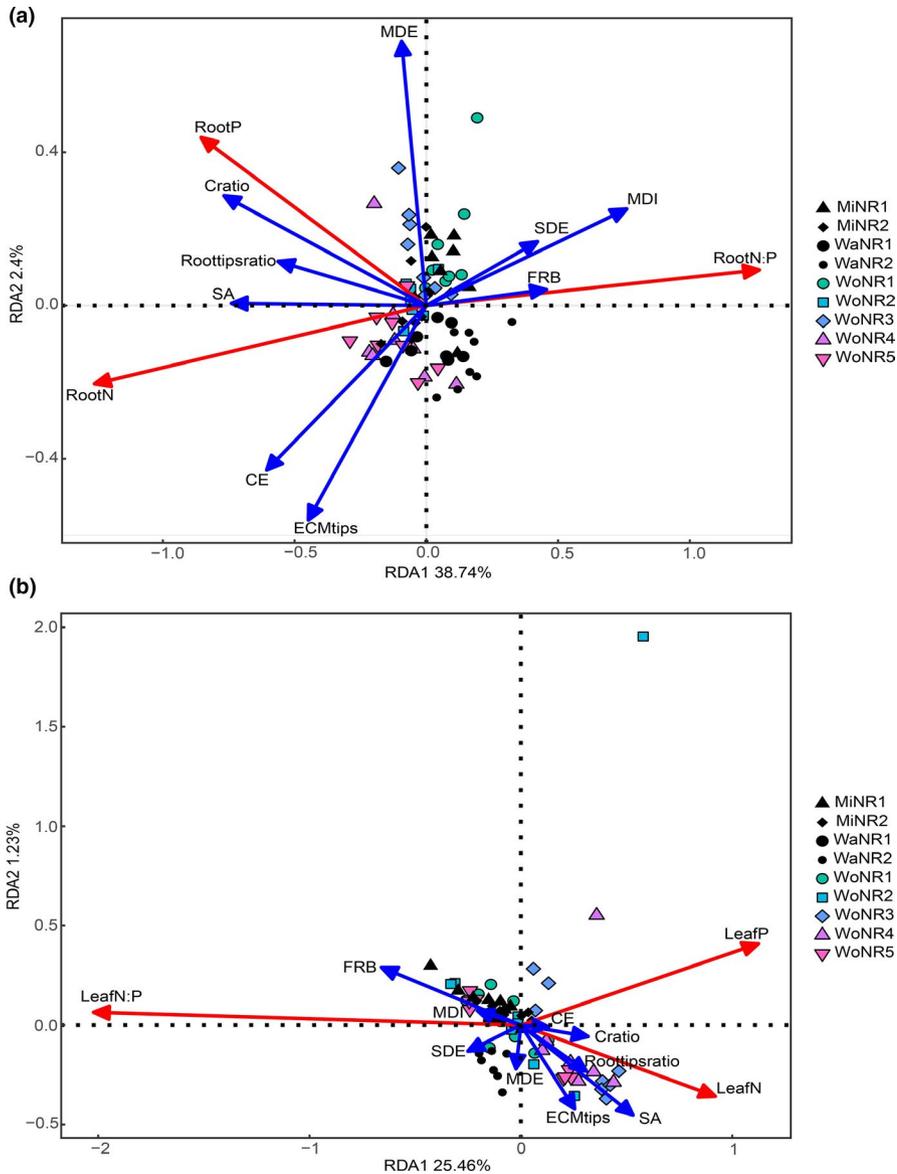
FIGURE 3 Redundancy analysis (RDA) ordination biplot of ECM traits and root nutrients (a) and foliar nutrients (b). C_{ratio}: Colonization ratio of ECM fungi; CE: ECM roots of contact exploration type; ECM_{tips}: ECM root tips per unit root biomass; FRB: fine root biomass; MDE: ECM roots of medium-distance 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 of short-distance exploration type; MiNR1: sampling at 3,077 m *asl* in Miyaluo Nature Reserve; MiNR2: sampling at 3,612 m *asl* in Miyaluo Nature Reserve; WaNR1: sampling at 3,070 m *asl*. in Wanglang Nature Reserve; WaNR2: sampling at 3,150 m *asl* in Wanglang Nature Reserve; WoNR1: sampling at 2,850 m *asl* in Wolong Nature Reserve; WoNR2: sampling at 3,000 m *asl* in Wolong Nature Reserve; WoNR3: sampling at 3,194 m *asl* in Wolong Nature Reserve; WoNR4: sampling at 3,413 m *asl* in Wolong Nature Reserve; WoNR5: sampling at 3,593 m *asl* in Wolong Nature Reserve

(Figure 2). The ECM soil exploration types exerted differential impacts on root and foliar N concentrations and N:P ratio (Figure 4).

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

Concerning our first hypothesis that ECM strategies mediate the partiality of N and P nutrition in below- and aboveground tissues in *A. faxoniana* in response to environmental variations, we found distinct effects of ECM strategies on plant N and P elemental stoichiometry in roots and leaves under varying soil and climate conditions. In this study, the mature *A. faxoniana* trees were deficient in root P (Table 1, P concentration: 0.72 ± 0.13 mg/g) as well as in root and foliar *N* (values of N concentration < 10 mg/g and N:P ratio < 14). It is suggested that the values of N:P ratio < 14 or >16, respectively, indicate N limitation or P limitation in plants and that tissue P

concentration < 1 mg/g and tissue N concentration < 10 mg/g are considered as deficient of the nutrient (Güsewell, 2004; Güsewell & Koerselman, 2002; 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 (Vitousek et al., 2010; Walker & Syers, 1976). According to the results in this study, the variations of ECM traits in *A. faxoniana* affected more on root and foliar P concentrations than on N concentrations (Figures 1 and 2), 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 nutrient requirements of plants (Merrild et al., 2013). However, the priority in nutrient acquisition is frequently determined by the strategic choices of plant species under multiple element limitations. It has been demonstrated that the ECM symbiosis give priority to the uptake of P but not N when in deficient supplies under different experimental conditions (Almeida et al., 2019; Smith et al., 2011;
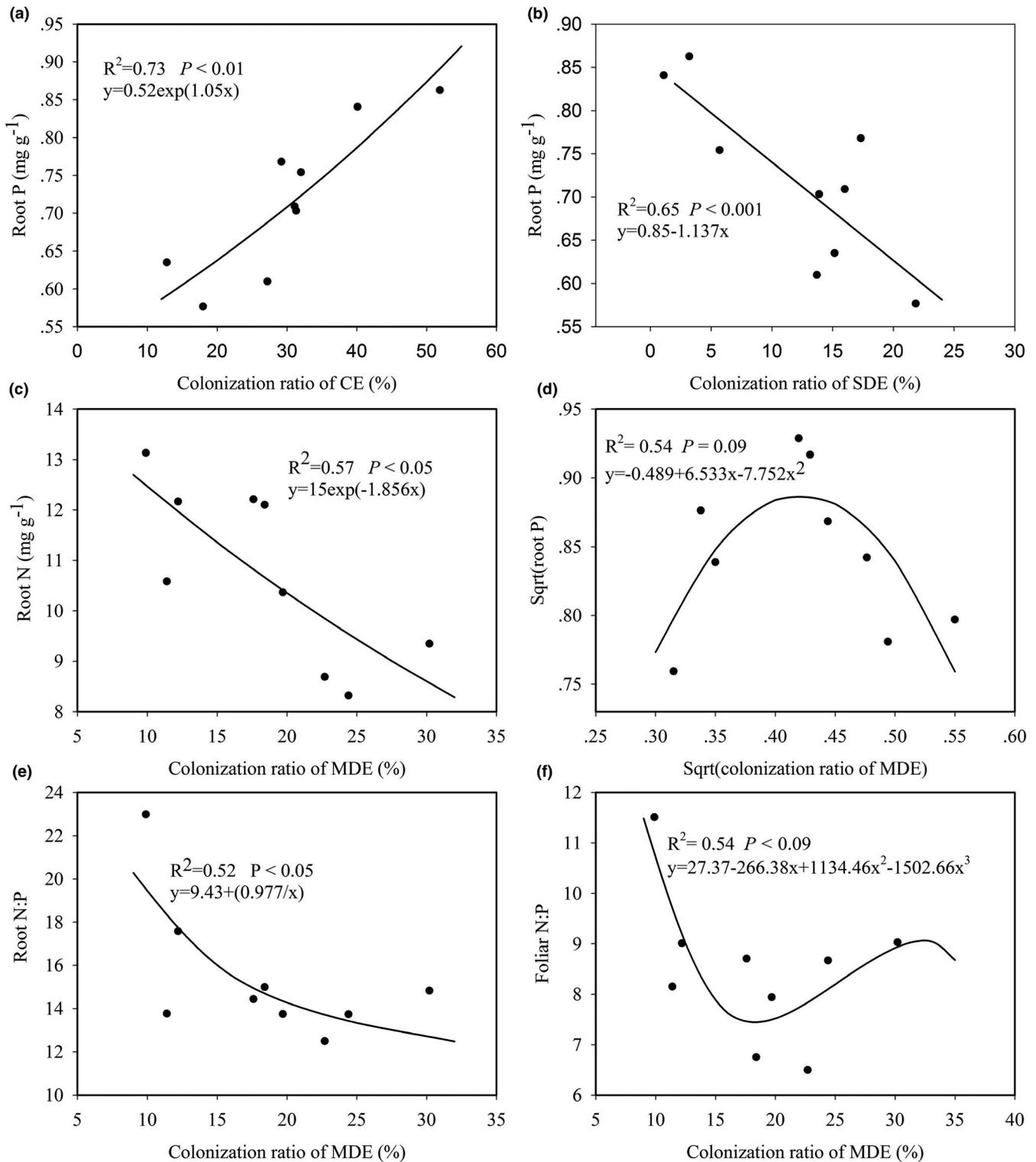
FIGURE 4 The significant relationships of root and foliar nutrients with soil exploration types. (a): the regressions of root P and the colonization ratio of CE; (b): the regressions of root P and the colonization ratio of SDE; (c): the regressions of root N and the colonization ratio of SDE; (d): the 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 of contact exploration type; SDE: ECM roots of short-distance exploration type; MDE: ECM roots of medium-distance exploration type

Zavišić et al., 2016). Moreover, it has been reported that the ECM symbioses sometimes do not largely alleviate N limitation (Franklin et al., 2014; Näsholm et al., 2013) 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).
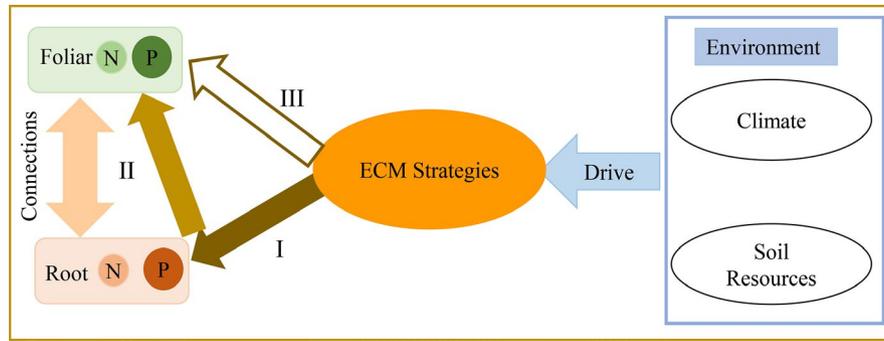
FIGURE 5 A conceptual model of the intervention of ECM symbiosis on root and foliar N and P in *Abies faxoniana*. I: The primary effects of ECM symbiosis on root nutrients. Root N and P nutrients are both strongly affected by ECM symbiosis, but the effects are stronger on root P than root N; II: indirect mediation of ECM symbiosis on foliar N and P driven by the nutrient limitation signals from leaves to roots; III: changes in foliar N and P caused by variations in ECM strategies. Changes in ECM foraging strategies impose greater influences on foliar P than on foliar N

The differential mechanisms of nutrient acquisition might change the nutrition preferences in plant species (Houlton et al., 2007; Zhang et al., 2018). Apart from soil resources and climate factors, our study shows that the varied ECM traits greatly influenced N and P nutrients in *A. faxoniana* (Figures 1 and 2). Overall, the ECM traits associated with 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 correlated to the below- and aboveground N and P concentrations in *A. faxoniana* (Figure 3). However, the fine root biomass and morphological diversity of ECM roots impacted negatively on the tissue N and P concentrations but positively on the N:P ratio, suggesting the trade-offs between the C investment for ECM root proliferation and morphology differentiation and the nutrients uptake. Accordingly, we draw the conclusion that both N and P nutrients of roots and leaves in *A. faxoniana* are primarily mediated 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. Research shows that the nutrient uptake efficiency of the symbiotic fungi in plants might mediate the concentration of the nutrients in roots and leaves, for example, the ECM colonization ratio, ECM absorption root vigor (Beltrano et al., 2013; Li et al., 2015; Vandenkoornhuyse et al., 2003), ECM root tip density, and absorptive capacity of ECM emanates (Ostonen et al., 2011), while the N and P stoichiometry in plant species could be affected by the function of mycorrhizal symbionts, for example, hyphae exploration ability and/or extracellular enzyme secretion (Chen et al., 2010). Plant nutrient uptake and balance exceedingly depend on the alternative foraging strategies of the ECM root systems (e.g., foraging precision of hyphae, morphology plasticity, and foraging range) under different environmental conditions (Chen et al., 2018; Einsmann et al., 1999; Köhle et al., 2018; Wang et al., 2006). 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; Craine et al., 2009; Hobbie & Hobbie, 2006), 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 varying soil and climate conditions, 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*

In confirmation to our second hypothesis that ECM soil exploration types differentially regulate the nutrient uptakes in host trees, 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 (Figures 2, 3a,b, and 4a) and negatively with that of the short-distance and the medium-distance exploration types (Figure 3a,b). Specifically, both root N and P concentrations were negatively associated with the frequency of short-distance exploration type, while a quadratic relationship was found between the root P concentration and the frequency of the medium-distance exploration type in a value range of 9%–30% (Figure 4b–d). Clearly, variations in the length of emanates in *A. faxoniana* in response to varying environmental conditions was not explainable by adjustment in nutrient uptake capacity. This contradicts the findings of positive relationships between the nutrient status of host plants and the length of emanates of ECM roots in literature (Agerer, 2001; Brandes et al., 1998; Hobbie & Agerer, 2010; Lilleskov et al., 2011). A probable explanation for this contradiction is that, in natural ecosystems, while ECM symbionts respond to soil resource deficiency in the way of root proliferation and production of emanating hyphae, the consequence of improved nutrition in host plants could be offset by increased energy cost for the ECM root systems under multiple resource limitation. In this study, the soil N and P were mostly deficient across the study sites (alkaline N: 40.78 ± 18.83 mg/g, total

P: 0.87 ± 0.26 mg/g). It is likely that the contact exploration type and the short-distance exploration type mediated 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). Ostonen et al., (2007), Ostonen et al., (2011) noted that host trees can rely on the high efficiency of resource capture of the root–mycorrhiza continuum while 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 themselves in priority while the host tree remains 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 C allocation to ECM emanates and nutrient uptake in host plants (Johnson et al., 2013; Magyar et al., 2007).

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 5). 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.

CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
Lulu Chen: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); writing–original draft (equal). **Chao Jiang:** Formal analysis (equal); methodology (equal); resources (equal). **Xiangping Wang:** Resources (equal); supervision (equal); writing–review and editing (equal). **Qiuhong Feng:** Data curation (equal); resources (equal); writing–review and editing (equal). **Xingliang Liu:** Data curation (equal); resources (equal); writing–review and editing (equal). **Zuoxin Tang:** Formal analysis (equal); methodology (equal); writing–review and editing (equal). **Osbert Jianxin Sun:** Conceptualization (lead); funding acquisition (lead); methodology (equal); project administration (lead); resources (lead); supervision (lead); writing–review and editing (lead).

ETHICAL APPROVAL
Performance of this study did not involve any critical damage to plants and ecosystems at the sites. Permissions for access to study sites and sampling were granted by the local administrations and management offices.

DATA AVAILABILITY STATEMENT
Data supporting this research are available from the Dryad Digital Repository at: https://doi.org/10.5061/dryad.pvmcvdnkb.

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