RESEARCH ARTICLE

Extraradical hyphae exhibit more plastic nutrient-acquisition strategies than roots under nitrogen enrichment in ectomycorrhiza-dominated forests

Xiaomin Zhu[1,2,3] | Hans Lambers[4] | Wanji Guo[5] | Dongdong Chen[1] |
Zhanfeng Liu[2,3] | Ziliang Zhang[6] | Huajun Yin[1]

[1]CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province & China-Croatia "Belt and Road" Joint Laboratory on Biodiversity and Ecosystem Services, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China

[2]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Coastal Zones, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

[3]South China National Botanical Garden, Guangzhou, China

[4]School of Biological Sciences, University of Western Australia, Perth, Western Australia, Australia

[5]Sichuan University, Chengdu, Sichuan, China

[6]Institute for Sustainability, Energy, and Environment, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Correspondence

Huajun Yin, CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province & China-Croatia "Belt and Road" Joint Laboratory on Biodiversity and Ecosystem Services, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China.
Email: yinhj@cib.ac.cn

Ziliang Zhang, Institute for Sustainability, Energy, and Environment, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.
Email: ziliangz@illinois.edu

Abstract

Ectomycorrhizal (ECM) functional traits related to nutrient acquisition are impacted by nitrogen (N) deposition. However, less is known about whether these nutrient-acquisition traits associated with roots and hyphae differentially respond to increased N deposition in ECM-dominated forests with different initial N status. We conducted a chronic N addition experiment ($25\,kg\,N\,ha^{-1}\,year^{-1}$) in two ECM-dominated forests with contrasting initial N status, that is, a *Pinus armandii* forest (with relatively low N availability) and a *Picea asperata* forest (with relatively high N availability), to assess nutrient-mining and nutrient-foraging strategies associated with roots and hyphae under N addition. We show that nutrient-acquisition strategies of roots and hyphae differently respond to increased N addition. Root nutrient-acquisition strategies showed a consistent response to N addition, regardless of initial forest nutrient status, shifting from organic N mining toward inorganic N foraging. In contrast, the hyphal nutrient-acquisition strategy showed diverse responses to N addition depending on initial forest N status. In the *Pinus armandii* forest, trees increased belowground carbon (C) allocation to ECM fungi thus enhancing hyphal N-mining capacity under increased N availability. By comparison, in the *Picea asperata* forest, ECM fungi enhanced both capacities of P foraging and P mining in response to N-induced P limitation. In conclusion, our results demonstrate that ECM fungal hyphae exhibit greater plasticity in nutrient-mining and nutrient-foraging strategies than roots do in response to changes of nutrient status induced by N deposition. This study highlights the importance of ECM associations in tree acclimation and forest function stability under changing environments.

1 | INTRODUCTION

Nitrogen (N) and phosphorus (P) are two essential nutrients that frequently limit plant growth and primary productivity in terrestrial ecosystems (Du et al., 2020; Elser et al., 2007). Dependent on environmental conditions, plants have evolved diverse nutrient-acquisition strategies (Lambers et al., 2008; Wen et al., 2022). In addition to root-foraging strategy by adjusting root physiological and morphological traits (Chen et al., 2016; Ma et al., 2018), plants also transfer photosynthetic carbon (C) into the rhizosphere via root exudation to stimulate mineralization of soil organic matter (SOM) (i.e., nutrient-mining strategy) (Dakora & Phillips, 2002; Dijkstra et al., 2013). Root nutrient-foraging and nutrient-mining processes both require C investment but target different pools of resources (e.g., labile vs. recalcitrant; inorganic vs. organic) (de la Riva et al., 2021; Roumet et al., 2016). Therefore, there is a trade-off between these two processes following changes of belowground resource allocation and soil nutrient supply (Meier et al., 2020; Wen et al., 2019; Williams et al., 2021). For examples, in low-nutrient environments, plants are more dependent on supplying abundant labile organic C (i.e., root exudates) to soil microbes that accelerate organic N mineralization by triggering rhizosphere priming (Cheng et al., 2014; Finzi et al., 2015). Conversely, in high-N environments, with lower C allocation to nutrient mining, modifying root architecture and morphology tends to be a dominant strategy to enhance root N foraging (de la Riva et al., 2021; Ma et al., 2018).

Ectomycorrhizal (ECM) associations are critical in mediating nutrient dynamics in temperate and boreal forests (Smith & Read, 2008; Steidinger et al., 2019). Within such symbiotic associations, the extraradical hyphae (hereafter, hyphae) emanating from ECM root tips provide growth-limiting nutrients (e.g., N and P) to host plants in exchange for C that supports fungal symbionts (van der Heijden et al., 2015). Like roots, the hyphae also form two major trait dimensions for nutrient acquisition. One dimension is related to the hyphosphere priming effect induced by hypha-derived C inputs beyond the root-accessible zone (i.e., hyphal nutrient-mining strategies) (Terrer et al., 2018; Wallander et al., 2011). The other dimension is characterized by adjusting hyphal functional traits to maximize nutrient foraging (i.e., hyphal nutrient-foraging strategies) (Agerer, 2001; Teste & Laliberté, 2019). ECM fungal taxa display considerable plasticity (i.e., intraspecific variation) in morphological or physiological traits in various environmental conditions (van der Linde et al., 2018; Suz et al., 2021). ECM traits such as exploration types, which bridge the morphology and differentiation of ECM hyphae to variation in nutrient-acquisition strategies, are good predictors of ecosystem processes (Defrenne et al., 2019; Koide et al., 2014). Therefore, trade-offs between hyphal nutrient-mining

and nutrient-foraging strategies may also exist and contribute to efficient nutrient acquisition under changing environments. However, most studies on nutrient-acquisition strategies generally consider roots and hyphae as an integrated organ (i.e., the mycorrhiza), ignoring the morphological and physiological plasticity of hyphae in nutrient acquisition and ecological functions due to the challenge in distinguishing root and hypha-mediated ecological effects in the field (Bergmann et al., 2020; Chen et al., 2016; Wen et al., 2022).

Increasing atmospheric N deposition has profoundly impacted forest N status and nutrient stoichiometry worldwide (Matson et al., 2002). Chronic N deposition may gradually shift N-limited forests toward N saturation or even trigger P limitation (Aber et al., 1998; Peñuelas et al., 2013) which will greatly alter belowground nutrient-acquisition strategies mediated by roots and mycorrhizal fungi (Deng et al., 2016). Despite both of roots and hyphae playing important roles in nutrient acquisition, the foraging and mining strategies exhibited by them might differently respond to elevated N deposition. From a "C-cost for N-benefit" perspective, trade-offs between root nutrient-mining and nutrient-foraging strategies under elevated N deposition are affected by belowground C allocation. Belowground C allocation is considered to limit the ability of plants to simultaneously release large amounts of exudates for nutrient mining and regulate root morphology for nutrient foraging (Honvault et al., 2021; Wen et al., 2022). In terms of hyphal nutrient-acquisition strategies, however, considering the asynchronous changes of nutrient limitation between host plant and ECM fungi under elevated N deposition (Guo et al., 2021; Lilleskov et al., 2019), the responses of hyphal traits might be more complicated, with the traditional cost–benefit relationship decoupled. For example, when N addition mainly mitigates N limitation of host plant, more photosynthate C will be allocated to aboveground growth, rather than to ECM fungi, leading to a decline of hyphal biomass and exudation and a lower hyphosphere priming effect (Hasselquist et al., 2016). Alternatively, increased ECM fungal biomass and hyphal biomass has also been reported under N addition, with N-limitation of host plant remaining unrelieved (Clemmensen et al., 2006; Lilleskov et al., 2019). In addition, ECM fungi can cope with P-limitation induced by N deposition by promoting hyphal production or increasing the abundance of ECM fungi involving long-distance exploration types (Lilleskov et al., 2011). Consequently, elevated N deposition likely impacts the trade-off between nutrient-foraging and nutrient-mining strategies of hyphae in ways distinct from those of roots. However, direct evidence disclosing the trade-offs between nutrient-mining and nutrient-foraging strategies of roots versus hyphae in the context of elevated N deposition is scarce. This hinders our understanding of the mechanistic linkages among mycorrhizal functional traits, belowground nutrient-acquisition strategies, and aboveground productivity in forests under changing environments.

The montane coniferous forests on the Qinghai-Tibet Plateau are characterized by plant growth and productivity being primarily limited by N, and trees are highly dependent on ECM symbionts for N acquisition (Du et al., 2020; Song et al., 2006). The current N deposition rate of local forest ecosystems is estimated at 10–20 kg N ha^{-1} year^{-1} (Zhu et al., 2015). This rate strongly exceeds N-deposition thresholds of ECM fungal communities (5–10 kg N ha^{-1} year^{-1}) and is expected to cause a decline in abundance, diversity, or functioning of ECM fungi (Pardo et al., 2011). However, to date, we lack a holistic understanding of the differential response of root and hyphal nutrient-mining and nutrient-foraging traits in ECM-dominated forests under chronic N deposition. In this study, we have performed a chronic N-addition experiment in two adjacent ECM-dominated coniferous forests since 2017, that is, a *Pinus armandii* forest and a *Picea asperata* forest. Our previous studies demonstrated that the *Picea asperata* forest has shifted toward P limitation, while the productivity of the *Pinus armandii* forest is still primarily N limited under chronic N addition (Yin et al., 2021; Zhang et al., 2022).

In this study, by taking advantage of the difference in nutrient limitation status between two coniferous forests, we aimed to examine whether trade-offs between nutrient-mining and nutrient-foraging strategies differ in roots and hyphae under N enrichment between two forests. We investigated nutrient-mining strategies of roots and hyphae by quantifying the root- and hypha-derived C inputs and their effects on nutrient mineralization using the ingrowth-core technique. We also characterized root and hyphal functional traits (e.g., root biomass, specific root length (SRL), root diameter, root branching number, and the relative abundance of ECM fungal hyphal exploration type) to assess effects of N addition on root and hyphal nutrient-foraging strategies. Specifically, we aimed to test the following hypotheses: (i) As N addition enhances soil N availability, plants are expected to reduce their relative investment on root production and root exudation (Fransson & Johansson, 2010; Kaiser et al., 2010). Thus, root-derived C inputs and associated root effects on nutrient mining would be reduced by N addition. Instead, roots are expected to depend more on foraging traits for efficient nutrient acquisition under N addition. (ii) Considering the asynchronous nutrient demand of host plants and ECM fungi (Prescott, 2022; Prescott et al., 2020) and the divergent variation in soil nutrient supply induced by N addition, hyphae would exhibit more plastic nutrient-acquisition strategies than roots.

2 | MATERIALS AND METHODS

2.1 | Study sites and the N-addition manipulation

The study sites were located at Tudiling Giant Panda National Park nearby the Maoxian Ecological Station of the Chinese Academic of Sciences (31°41′N, 103°53′E) in Maoxian country, Sichuan Province, China. According to the long-term meteorological monitoring record of the station, the local mean annual temperature, precipitation,

and evaporation are 8.9°C, 920 mm, and 796 mm, respectively. A chronic N-addition experiment was conducted in two adjacent ECM-dominated coniferous forests, a *Pinus armandii* forest and a *Picea asperata* forest, to disentangle the effects of chronic N deposition on the structure and function of these forests. These two forests were planted in the 1970s under the auspices of a local reforestation program. The soils in both forests are classified as a Cambic Umbrisol. Compared with the *Pinus armandii* forest, the *Picea asperata* forest has higher concentrations of soil organic C, total N, dissolved inorganic N (DIN, NH$_4^+$+NO$_3^-$), DIN: plant-available soil phosphorus (Av. P) ratio, and net N-mineralization rate but lower Av. P concentration ($p < .05$, Table S1). These results suggest that the *Pinus armandii* forest has relatively low N availability, but high soil P availability compared to the *Picea asperata* forest, providing a chance to explore whether nutrient-acquisition strategies of roots and hyphae respond differently to N addition due to changes in forest N and P status.

In April 2017, three replicated blocks of two N-treatment conditions (the control with 0 kg N ha^{-1} year^{-1} and N addition with 25 kg N ha^{-1} year^{-1}) were randomly established in each plantation (Figure 1a), and each block was at least 20 m apart. Two 10 m × 10 m plots separated by 10-m wide buffer strips were established in each block. The ammonium nitrate (NH$_4$NO$_3$) was divided into six doses and sprayed to each plot monthly from May to October (i.e., 41.6 g N per month) in each year. The N-fertilizer application has been going on since 2017.

2.2 | Ingrowth-core installation and collection

To partition the C inputs from roots and hyphae, three types of ingrowth cores (inner diameter 6 cm, height 15 cm) were set up in each plot in May 2017 as per Phillips et al. (2012) and Zhang et al. (2018): one with 2-mm mesh allowing the penetration of roots and hyphae (R-cores), one with a 48-μm mesh only permitting the penetration of hyphae (H-cores), and one with a 1-μm mesh that prevents the ingrowth of roots and hyphae (EH-cores). The natural abundance of ^{13}C has been widely used to estimate changes in soil C stock. Commonly, C$_3$ plants are grown in soils with organic matter derived from C$_4$ plants (Keller et al., 2021; Kuzyakov & Domanski, 2000; Wallander et al., 2011). Six sets of ingrowth cores with 2-mm, 48-μm and 1-μm mesh-size were filled with homogeneous maize-grown soil and installed in the topmost mineral horizon in each plot (Figure 1b). These cores were used to quantify the amount of root- and hypha-derived C input. The C isotopic signature (δ^{13}C) in the maize-grown soil was −24.29 ± 0.04‰, which was significantly enriched in ^{13}C compared with the C$_3$ roots (−27.14% to −27.85%) and hyphae (−26.79% to −28.55%) in two forests (Table S3). In addition, six sets of ingrowth cores of different mesh sizes were filled with homogeneous native mineral soil (0–15 cm) collected from each plot in each forest and installed in the same soil horizon to assess the effects of roots and hyphae on N- and P-acquisition enzyme activities (Figure 1b). Before filling the cores, all C$_4$ and native soils were sieved through
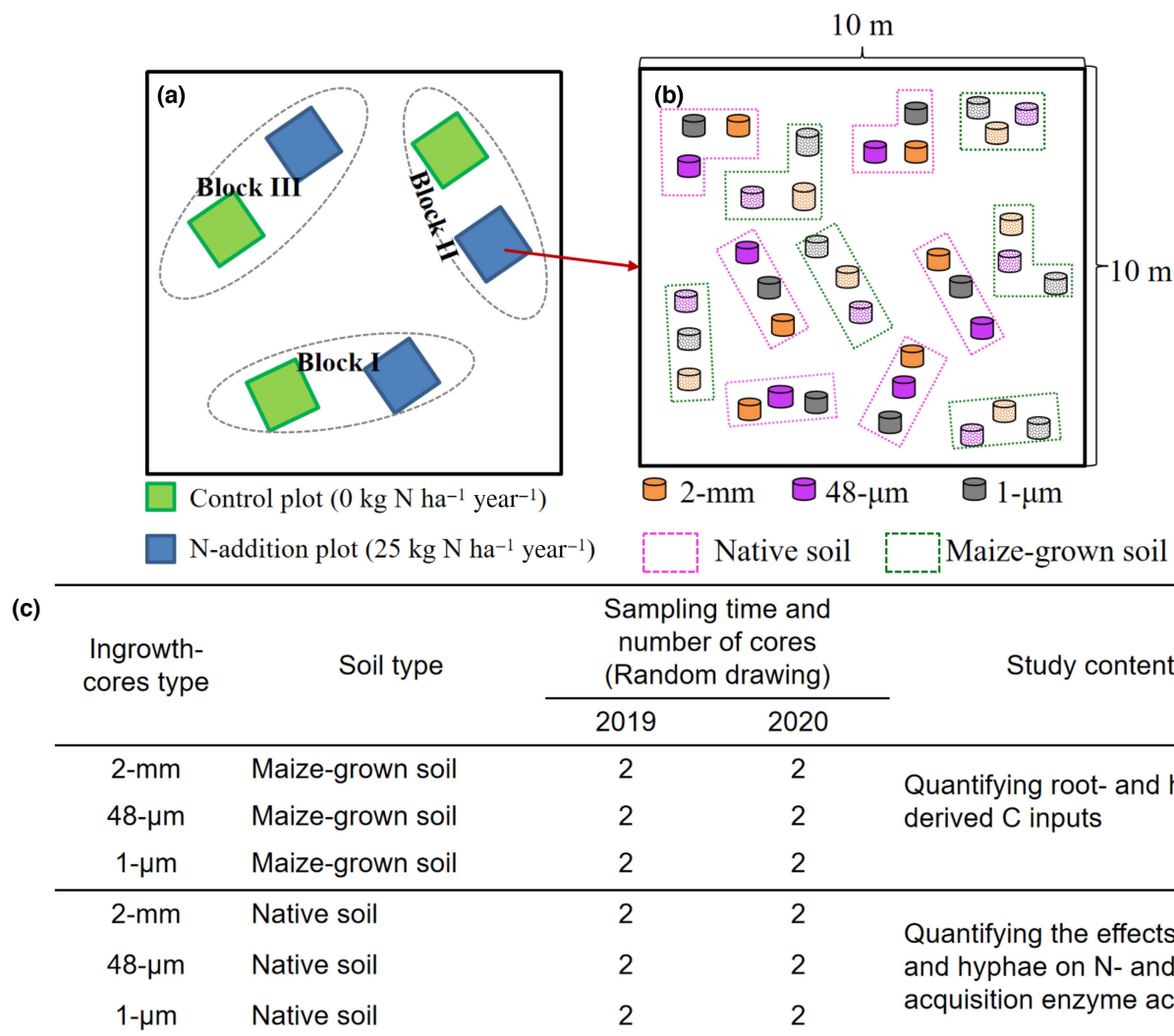
FIGURE 1 A schematic diagram illustrating the experimental design of the nitrogen (N)-addition treatment, installment of ingrowth cores and the sampling procedure. (a) Control and N-addition plots in each forest; (b) arrangement of in-growth cores with 2-mm, 48-μm, and 1-μm mesh size in each plot; (c) sampling time and frequency of in-growth cores in each plot. [Colour figure can be viewed at wileyonlinelibrary.com]

a 5-mm mesh after removing visible roots. Each ingrowth core was at least 20 cm apart. As the top of the cores were tied tightly (preventing entry of fresh litter), the C source in the R-cores was mainly derived from roots, hyphae, and litter leachates, and that of the H-cores was from hyphae and litter leachates, while the EH-cores received C only from litter leachates. To block the entry of new C derived from saprophytic hyphae outside the cores, we spread a 2-mm-thick layer of silica sand (0.36–2.0 mm), 99.6% silicon dioxide (SiO_2) around the cores. In a preliminary experiment, we found that more than 80% of the fungal taxa entering silica-sand-blocked cores from outside belong to ECM fungi (the relative abundance at trophic-Mode level, %), while saprophytic mycorrhizal fungi only account for 4%–15% (Unpublished data). This result again confirmed that silica sand as a barrier for the entry of external saprophytic fungal hyphae in previous study (Hagenbo et al., 2017). In addition, we used mesh cores (inner diameter 6 cm, height 15 cm, 48 μm mesh size) containing 135 g acid-washed silica

sand to harvest and quantify ECM fungal hyphal biomass (Guo et al., 2021; Wallander et al., 2001).

We harvested ingrowth cores in August 2019 and August 2020, respectively. Two sets of cores filled with C_4 soil and native soil were randomly collected from each plot at each sampling date (Figure 1c). In total, four sets of cores filled with C_4 soil and native soil were collected during the experimental period, and the remaining ingrowth cores in the plots were reserved for a follow-up study. Cores were transported to the laboratory using an ice box. The C_4 and native soil inside the cores with the same size of mesh in each plot were sieved through a 2-mm mesh and thoroughly mixed to form a composite sample for analysis, respectively. The total number of soil samples per treatment was six for C_4 soils and six for native soils for two sampling dates (2019 and 2020) in each forest. The C_4 soils were air-dried for the determination of soil organic C (SOC) and $\delta^{13}C$. Subsamples of the native soils inside the cores were stored at 4°C for the analyses of soil

nutrient availability and extracellular enzyme activities within 48 h of sampling.

2.3 | Nutrient-foraging traits of roots and hyphae

Fine root/hyphal biomass, root morphology, and the relative abundance of ECM fungi with different hyphal exploration type were determined to access the changes of nutrient-foraging strategies of roots and hyphae under N addition. Fine roots inside the 2000-µm mesh cores were manually picked out and washed thoroughly, and then scanned at 600 dpi with images analyzed using WinRHIZO (Regent Instruments, Inc.) to record root diameter, accumulative root length, and root-branching numbers. After scanning, the root samples were oven-dried at 60°C for 48 h to determine fine root biomass. SRL was calculated as total root length per unit root dry mass (cm g^{-1}). The hyphae were collected using the suspension-filtration method (Wallander et al., 2004), subsequently lyophilized and stored at −20°C (Guo et al., 2021). To quantify the hyphal biomass in silica sand samples, the fungal-specific biomarker (ergosterol) was extracted as per Wallander and Nylund (1992) and quantified using high-performance liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific). Detailed descriptions of laboratory analyses are shown in Appendix S1. In our previous study, we characterized the response of ECM fungal community composition to N addition in the same forests (Guo et al., 2021). Details of soil DNA extraction, amplicon barcoding, and sequence data processing are described in Appendix S1 and Guo et al. (2021). The relative abundance of fungal communities at trophic-mode level and the relative abundance of ECM fungal community at genus level under the control and N-addition treatments in two forests are shown in Figure S1. In total, 32 and 27 ECM fungal genera were identified in the *Pinus armandii* and *Picea asperata* forests, respectively. Among them, 16 ECM fungal genera were shared between the two forests which accounted for 81.7% and 99.6% of the ECM fungal community in each forest (Figure S1). In this study, we reclassified ECM fungal hyphal exploration types into contact-short distance (C-S), contact-medium distance (C-M) type, and medium-long distance (M-L) types, based on Agerer (2001, 2006) and Tedersoo and Smith (2013) to reflect changes in hypha foraging strategy.

2.4 | Nutrient-mining traits of roots and hyphae

The amount of root- and hypha-derived C inputs as well as the root effect (RE) and hypha effect (HE) on N- and P-acquisition enzyme activities were determined to access the changes of nutrient-mining strategies of roots and hyphae under N addition. Briefly, the C and N concentrations and δ^{13}C of the roots, hyphae, and C$_4$ soil in cores were determined on a continuous flow CN analyzer (Flash EA 2000; Thermo Fisher Scientific) coupled to an isotope mass spectrometer (model DeltaV; Thermo Fisher Scientific). The C isotope ratio values were expressed with the delta notation (δ):

$$\delta^{13}C\%_o = \left[\left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000\right],$$

where R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ ratio of samples and standard, respectively, and $R_{standard}$ is referenced to the Vienna Pee Dee Belemnite (0.13‰). The ^{13}C isotopic signature and C concentration of C4 soil in each type of ingrowth core are shown in Table S2. Root- and hypha-derived C inputs into each core were quantified using a two-source isotopic mixing model as per Panzacchi et al. (2016):

$$f_{new} = \frac{\delta^{13}C_{R/H} - \delta^{13}C_{EH}}{\delta^{13}C_{root/hypha} - \delta^{13}C_{EH}},$$

$$\text{New C input} = SOC_{R/H} \times f_{new},$$

where $\delta^{13}C_{R/H}$ is the $\delta^{13}C$ of the soil in the R- or H-cores, $\delta^{13}C_{EH}$ is the $\delta^{13}C$ of the soil in the EH-cores, and $\delta^{13}C_{root/hypha}$ is the $\delta^{13}C$ of the root or hypha (Table S3). $SOC_{R/H}$ is the %C of the soil in the R- or H-cores. The amount of new C originating from root was calculated by the difference between new C inputs in the R-cores and that in the H-cores.

The activities of two N-acquisition enzymes (leucine aminopeptidase (LAP) and 1,4-β-N-acetyl-glucosaminidase) and one P-acquisition enzyme (acid phosphatase) were measured to evaluate the mineralization of soil organic N and soil organic P, respectively. The enzyme activities were assayed using a microplate fluorometric method (Saiya-Cork et al., 2002). To assess the influences of roots and hyphae on soil nutrient mineralization, the RE and HE were calculated as the ratios of enzymatic parameters in the R-cores to its corresponding values in the H-cores (R$_{core}$/H$_{core}$) and the ratios of enzymatic parameters in the M-cores to its corresponding values in the EH-cores (H$_{core}$/EH$_{core}$), assuming that the RE and HE are additive in the R-core (Finzi et al., 2015). Details of laboratory protocols and calculations are shown in Appendix S1.

2.5 | Statistical analyses

The Shapiro–Wilk and HOVTEST tests were used to check for the assumptions of normality and homogeneity of variances, respectively. One-way analysis of variance (ANOVA) with mixed linear models followed by Tukey's HSD post-hoc test was performed to test the effect of N addition on root and hyphal traits including root and hyphal biomass, SRL, root diameter root branches number, root-derived and hypha-derived C inputs, and their effects on enzyme activities with N-treatment as a fixed factor, and block as a random factor. Linear regression analyses were conducted between root- and hypha-derived C inputs and the size of RE and HE on N- and P-acquisition enzyme activities to assess the coordinated variation of C-inputs and nutrient mineralization. A non-metric multidimensional scaling analysis (NMDS) with PERMANOVA analysis was conducted to test the difference in ECM community composition at genera level between the control and N-addition treatment (Figure S1). One-way ANOVA followed by Tukey's HSD post-hoc test was performed to test the

effects of N addition on the relative abundance of ECM fungi at generic level and hyphal exploration types. Furthermore, a principal component analysis (PCA) with PERMANOVA analysis was performed to test the trade-offs between nutrient-mining and nutrient-foraging traits adopted by roots and hyphae under the control and N-addition treatments in two forests. Data of root- and hyphal traits used in the PCA analysis were measured in 2019 and 2020, and the relative abundance of ECM fungi in different exploration types was only determined in 2019. To assess the N-effect on the main axes of variations of root and hyphal traits, we performed one-way ANOVA with the PCA scores of the first components (PC1) as the dependent variables and the N treatment as the factor (Table S6). Post-hoc Tukey tests were performed to check the significance of pairwise differences between the control and N-addition plots. Pearson correlation analysis was used to examine the correlations among root- or hyphal traits as well as between root and hyphal traits and the scores of PC1. The ANOVA analyses were conducted with the package SAS v.9.4 (SAS Institute Inc.). The PCA and NMDS analyses were performed using the "PCA" and "NMDS" function of the stats package (v. 1.50) in Origin software. Origin 2021 (OriginLab Co.) was used to generate figures. The significance level for all statistical tests was set at $p < .05$.

3 | RESULTS

3.1 | Changes in "C input-nutrient mining" traits of roots and hyphae under N addition

The responses of root-derived C input to N addition were consistent across two forests, showing a decrease of 0.76 mg C g^{-1} and 1.05 mg C g^{-1} relative to the controls in the *Pinus armandii* and the *Picea asperata* forests, respectively ($p < .05$, Figure 2a; Table S3). However, the responses of hypha-derived C input greatly varied between the two forests, showing an increase of 0.89 mg C g^{-1} in the N-addition plots compared with the controls in the *Pinus armandii* forest ($p < .05$), with no significant difference in the *Picea asperata* forest (Figure 2d; Table S3). In the *Pinus armandii* forest, the activities of N-acquisition enzymes of the soil in the R- and H-cores were significantly increased by 14.6 nmol g^{-1} soil h^{-1} and 33.3 nmol g^{-1} soil h^{-1}, respectively, in the N-addition plots compared with the controls ($p < .05$, Table S5). Accordingly, N addition changed the RE and HE on activities of N-acquisition enzymes by −31% and +134% in this forest, respectively (Figure 2b,e; Table S3). However, the activities of N-acquisition enzymes of the soil in each type of ingrowth cores were not significant changed by N addition in the *Picea asperata* forest (Table S5). The activities of P-acquisition enzymes in the R- and H-cores were significant increased by N addition in both forests, with an increase of 18.8 and 31.5 nmol g^{-1} soil h^{-1} in the *Pinus armandii* forest and an increase of 74 and 85.5 nmol g^{-1} soil h^{-1} in the *Picea asperata* forest, respectively ($p < .05$, Table S5). Accordingly, compared to the control plots, the RE on P-acquisition enzymes activity in the N addition plots was decreased by −41% and −43%, and the HE on activities

of P-acquisition enzymes in the N addition plots was increased by +68% in the *Pinus armandii* and *Picea asperata* forests, respectively (Figure 2c,f; Table S3). Linear regression analysis showed that both root- and hypha-derived C inputs, in most cases, were significantly and positively correlated with the size of RE and HE on activities of N-and P-acquisition enzymes, respectively. Specifically, the RE on N-acquisition enzyme activities was significantly decreased with the decline of root-derived C input in the *Pinus armandii* forest, while the RE on P-acquisition enzyme activities was significantly decreased with the decline of root-derived C input under N addition in both forests (Figure 2b,c). By comparison, the HE on activities of N- and P-acquisition enzymes was increased with the increase in hypha-derived C input under N addition in the two forests (Figure 2e,f). In addition, the slopes of regression curves showed that the increase in the HE on N-acquisition enzyme activities was steeper than that of HE on P-acquisition enzyme activities in the *Pinus armandii* forest, while the pattern was the opposite in the *Picea asperata* forest (Figure 2e,f).

3.2 | Changes in nutrient-foraging traits of roots and hyphae under N addition

The responses of nutrient-foraging traits of roots (SRL, root diameter, and root-branching numbers) and ECM fungal hyphae (hyphal exploration type) to N addition were different between the two forests (Figure 3). Specifically, in the *Pinus armandii* forest, the SRL was increased by 146.3 cm g^{-1} ($p = .04$), and root diameter was decreased by 0.23 mm ($p = .02$) in the N-addition plots relative to the controls. In the *Picea asperata* forest, the numbers of root branches was increased by 2816 g^{-1} ($p = .02$) in the N addition plots compared to the control plots, and no such significant change was found in the SRL and root diameter (Table S3). The relative abundance of ECM fungal were 30% and 11% in the control plots in the *Pinus armandii* forest and the *Picea asperata* forest, respectively. The relative abundance of ECM fungi was significantly increased to 60% under N-addition in the *Pinus armandii* forest, but that in the *Picea asperata* forest was not affected by N addition (Figure S1). In terms of hyphal exploration types, N addition significantly increased the relative abundance of C-S and C-M explorers (*Russula*, *Tuber*, and *Inocybe*) and decreased the abundance of M-L explorers (*Amphinema* and *Rhizopogon*) in the *Pinus armandii* forest (Figure 4a,b). In contrast, N addition significantly increased the relative abundance of M-L explorers (*Amphinema*), but decreased the relative abundance of C-S and C-M explorers (*Tomrntella*, *Trichopaea*, *Inocybe*, and *Lactifluus*) (Figure 4c,d).

3.3 | Correlations of nutrient-mining and nutrient-foraging traits of roots and hyphae under N addition

The PCA showed that PC1 explained 54%–76% of the total variance in functional traits of roots and hyphae measured between
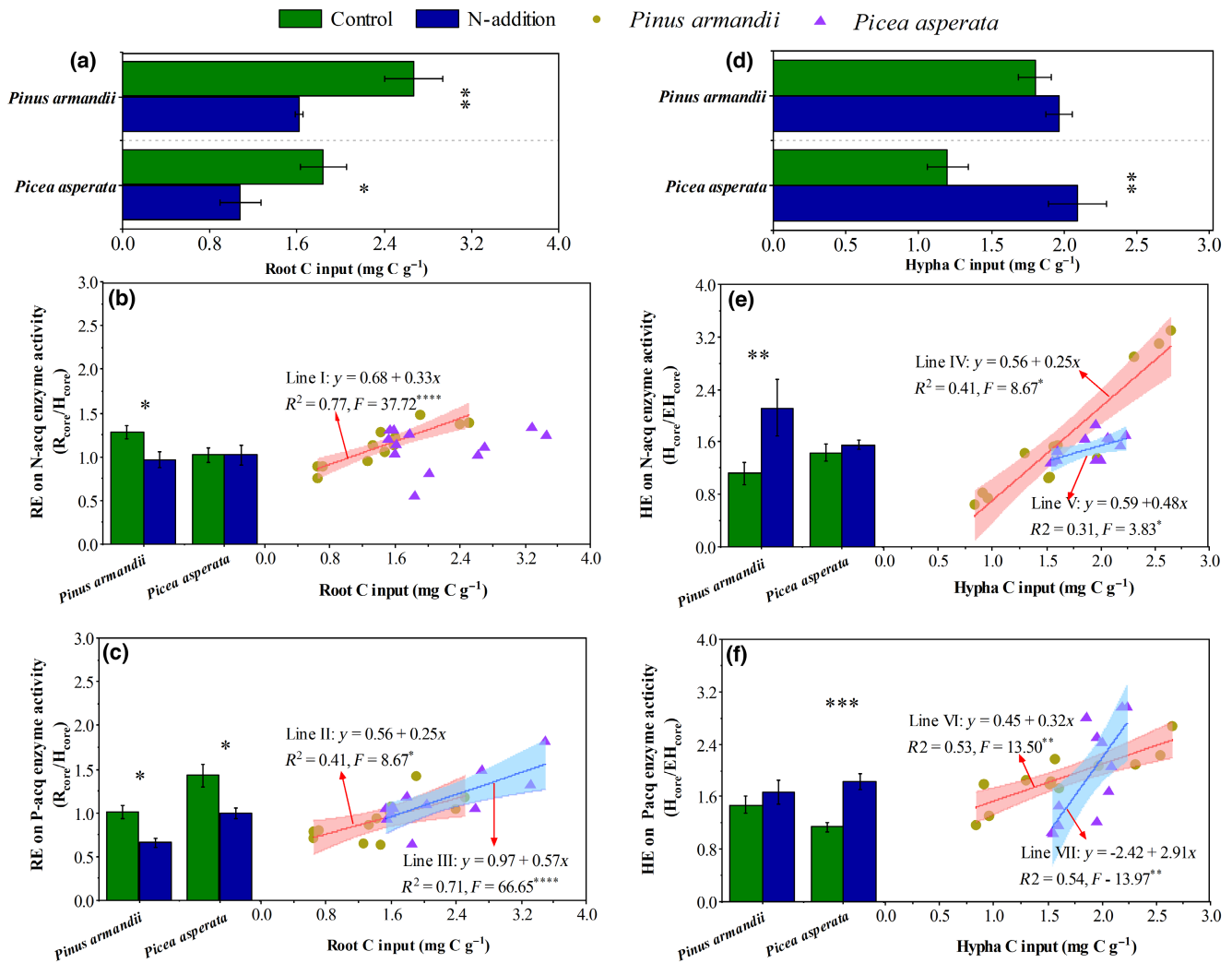
FIGURE 2 Root- and hypha-derived C inputs and their effects on nutrient-mining under the control and N addition treatments in two ECM-dominated forests. (a) and (d) show the effect of N addition on root- and hypha-derived C inputs in the *Pinus armandii* and *Picea asperata* forests, respectively. (b) and (c) show the size of root effect (RE) on the activities of N-acquisition and P-acquisition enzymes and their correlation with root-derived C inputs; (e) and (f) represent the hypha effect (HE) on the activities of N-acquisition and P-acquisition enzymes and their correlation with hypha-derived C inputs. The RE and HE on soil enzyme activities are indicated by the ratios of enzymatic parameters in the R-cores to their corresponding values in the H-cores, and the ratios of enzymatic parameters in the H-cores to their corresponding values in the EH-cores, respectively. RE and HE >1 indicates that roots and hyphae have a positive effect on enzyme activities, while RE and HE <1 denotes a negative effect. The data in bar plots are the mean values of two sampling dates (2019 and 2020), $n = 6$. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. Lines and areas in red are the fitting curves and 95% confidence intervals of the *Pinus armandii* forest, respectively. Lines and areas in blue are the fitting curves and 95% confidence intervals of the *Picea asperata* forest, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

the control and N-addition plots (Figure 5). For roots, PC1 was positively correlated with root-derived C input, the RE on activities of N- and P-acquisition enzymes and root diameter but negatively with SRL in the *Pinus armandii* forest (Figure S2a). Similarly, the PC1 was positively correlated with root-derived C input, the HE on P-acquisition enzyme activity and root biomass but negatively with root-branching numbers in the *Picea asperata* forest (Figure S2b). The one-way ANOVA indicated that the PC1 of root traits in both forests was separated by N treatment ($p < .05$, Table S6). Compared with those in the control plots, root functional traits in the N-addition plots were characterized by higher SRL (*Pinus armandii* forest) or

greater root branching (*Picea asperata* forest) but a lower RE on soil N-acquisition enzyme activity and root-derived C input in both forests (Figure 4a,b).

For hyphae, the PC1 was positively correlated with hypha-derived C input, the HE on N-acquisition enzyme activity, hyphal biomass, and the abundance of C-S and C-M distance explorers but negatively with the abundance of M-L distance explorers in the *Pinus armandii* forest (Figure S1). However, in the *Picea asperata* forest, the PC1 was positively correlated with the HE on P-acquisition enzyme activity and the abundance of M-L distance explorers but negatively with hyphal biomass and the abundance of C-S and C-M distance
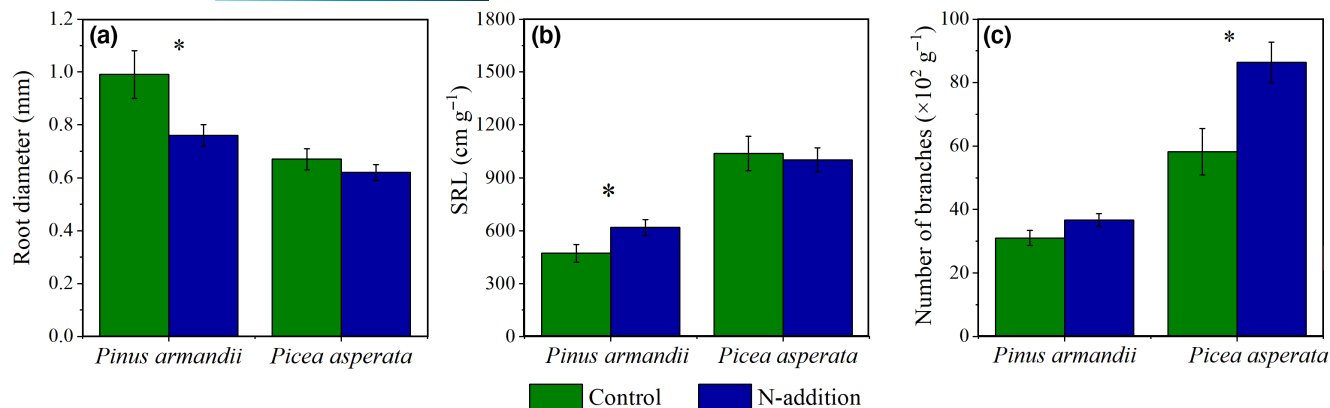
FIGURE 3 Changes in root functional traits including specific root length (SRL) (a), mean fine root diameter (RD) (b), and root-branching numbers (c), which partly represent the foraging capacity of fine roots. The data are the mean values over two sampling years (2019 and 2020). "*" denoted the difference between control and N-addition treatment was statistically significant at $p < .05$. [Colour figure can be viewed at wileyonlinelibrary.com]
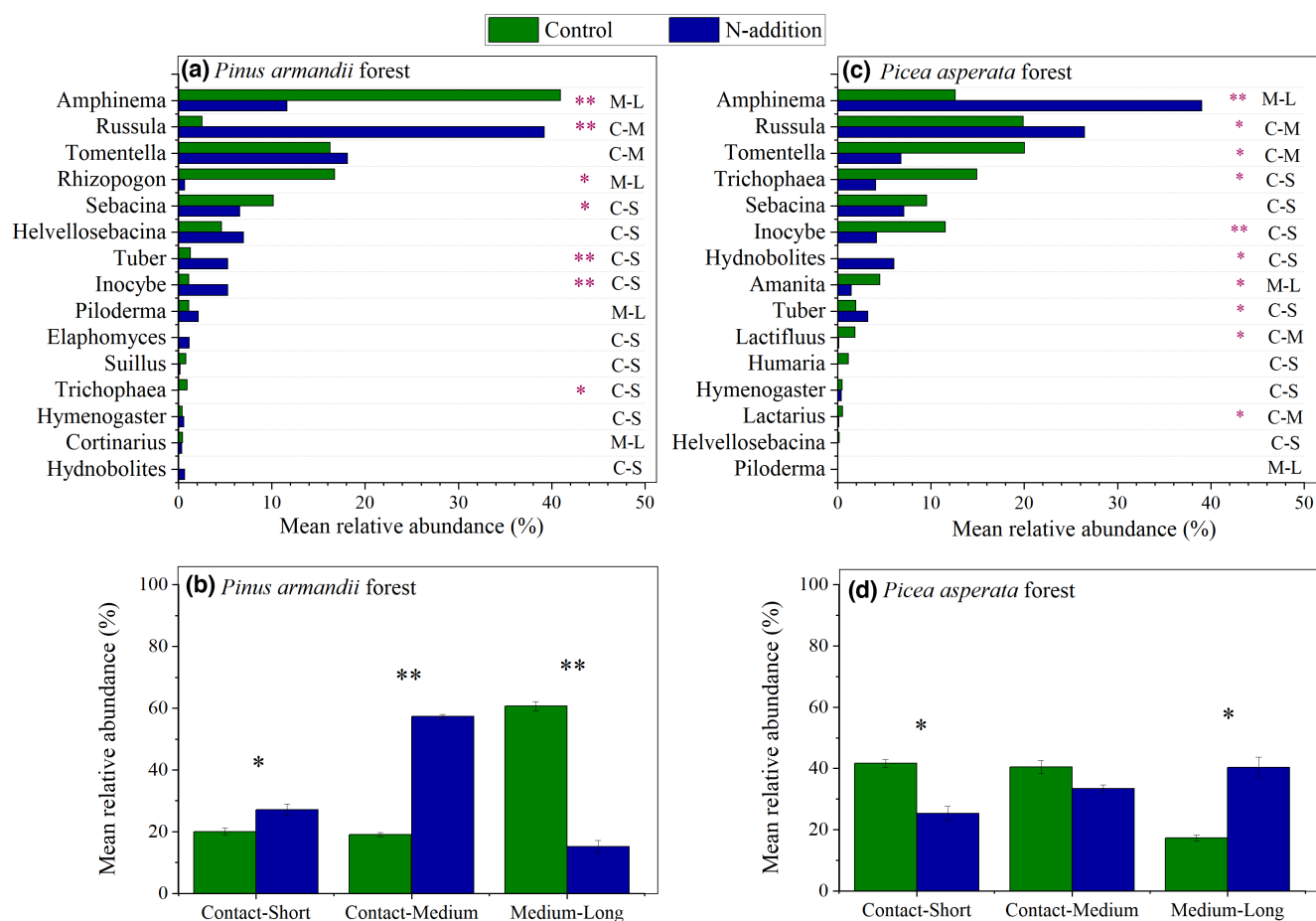

FIGURE 4 The relative abundance of the top 15 ectomycorrhizal (ECM) fungal genera (%) in soils of the control and N-addition plots (a, c) and the effect of N-addition on the relative abundance of ECM fungi in different hyphal exploration types (b, d) in the *Pinus armandii* forest and the *Picea asperata* forest. The relative abundance of ECM fungal genera were calculated the percentage (%) of the numbers of ECM fungal OTUs in genera level to the total ECM fungal OTUs numbers. *$p < .05$, **$p < .01$. [Colour figure can be viewed at wileyonlinelibrary.com]

explorers (Figure S1). The one-way ANOVA indicated that the PC1 of hyphal traits in both forests was separated by N treatment ($p < .01$, Table S6). In the *Pinus armandii* forest, hyphal functional traits in the

N-addition plots were characterized by greater hypha-derived C input and a greater HE on N-acquisition enzyme activity but greater abundance of C-S and C-M distance explorers compared with those
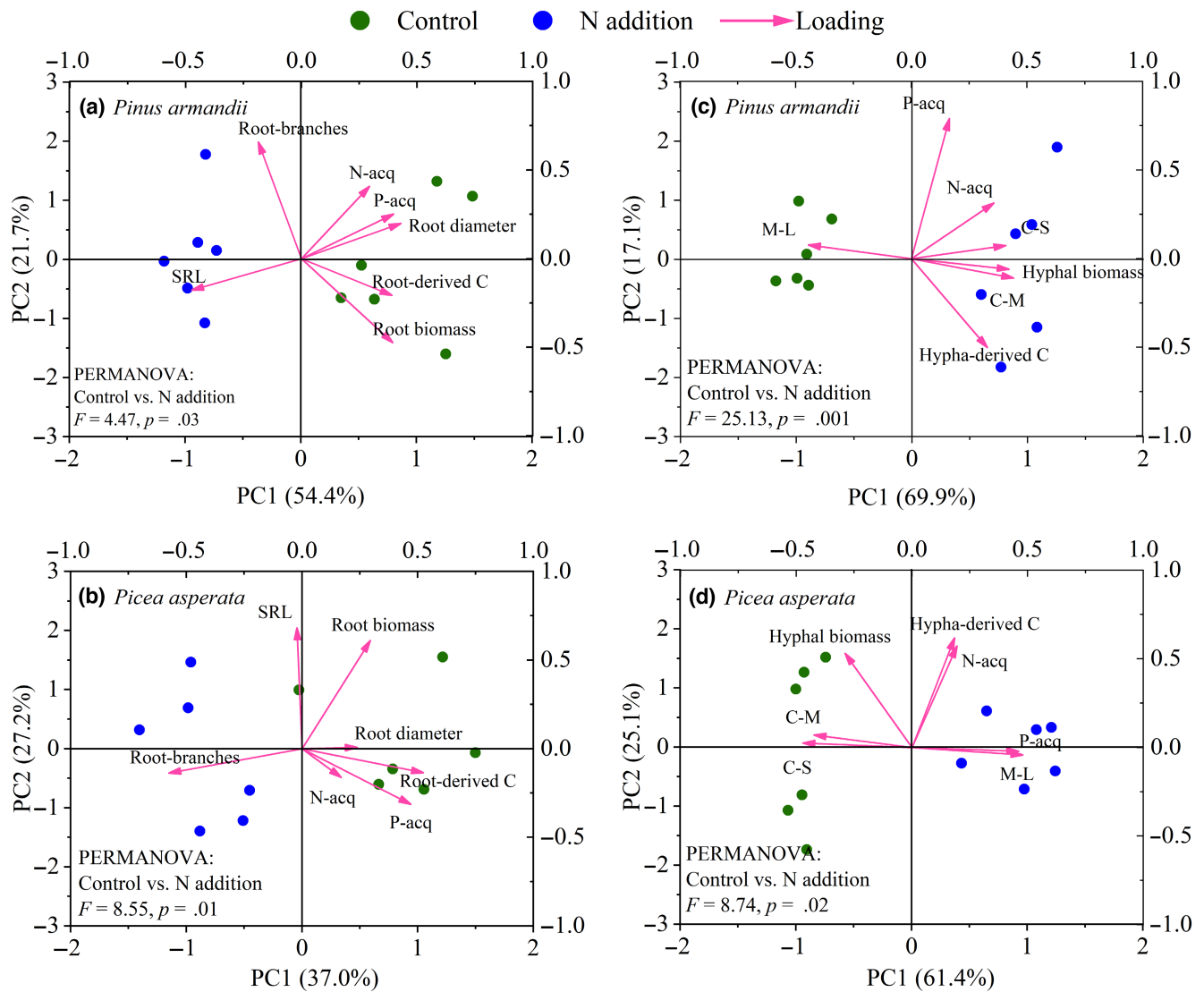
FIGURE 5 Principal component analysis (PCA) for the functional traits of roots (a, b) and hyphae (c, d) which characterize the nutrient-foraging strategy (fine root biomass, SRL, root diameter, root-branching numbers, hyphal biomass, ECM fungal hyphal exploration type) and the nutrient-mining strategy (root- or hypha-derived C input, root effect and hypha effect on activities of N- and P-acquisition enzymes). RB, root biomass; SRL, specific root length; RD, root diameter; N-acq, root effect or hypha effect on the activities of N-acquisition enzymes; P-acq, root effect or hypha effect on the activities of P-acquisition enzymes. C-S, the relative abundance of C-S explorers; C-M, the relative abundance of C-M explorers; M-L, the relative abundance of M-L explorers. [Colour figure can be viewed at wileyonlinelibrary.com]

in the control plots (Figure 5c). In the *Picea asperata* forest, hyphal functional traits in the N-addition plots were represented by a greater HE on P-acquisition enzyme activity and greater abundance of M-L distance explorers compared with those in the control plots (Figure 5d).

4 | DISCUSSION

Understanding trade-offs between nutrient-mining and nutrient-foraging strategies of mycorrhizal symbionts and how they respond to various environmental changes is fundamental to build a universal and comprehensive mycorrhizal symbiont trait-based framework in forests (Chaudhary et al., 2022; Wen et al., 2022). Our results

show that roots and hyphal nutrient-acquisition strategies respond differentially to N addition. Specifically, root nutrient-acquisition strategies showed a consistent response pattern in the two forests, shifting from mining soil organic N toward foraging soil inorganic N (Figure 6). In contrast, the response of hyphal nutrient-acquisition strategies to N addition varied between the two forests, depending on ecosystem N/P status and the nutrient demand of host trees and ECM fungi. We found an enhanced hyphal N-acquisition strategy for alleviating N limitation in the *Pinus armandii* forest with relatively low N status, but an enhanced hyphal P-acquisition strategy for coping with the N-induced P limitation in the *Picea asperata* forest with relatively high N status (Figure 5). Collectively, these results indicate that the nutrient-acquisition strategies exhibited by ECM fungal hyphae are more plastic than those of roots in response to chronic N
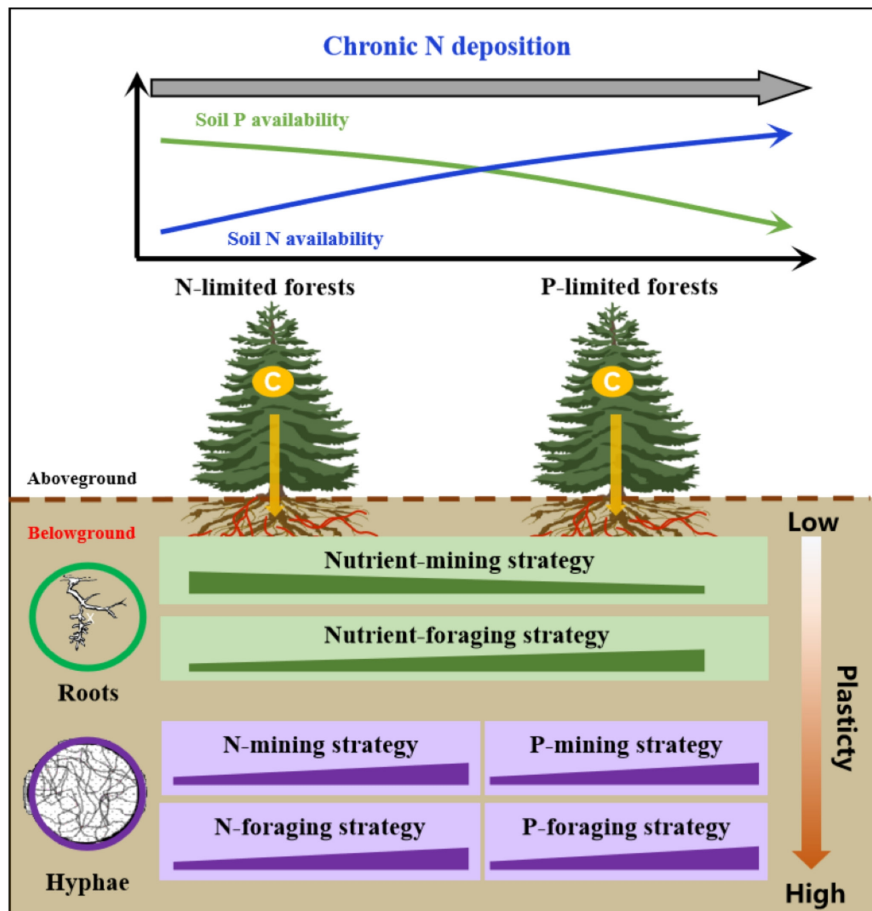
FIGURE 6 A conceptual framework showing the possible differences in trade-off between nutrient-mining and nutrient-foraging strategies exhibited by roots and hyphae in ECM-dominated coniferous forests when nutrient status shifts from N- to P-limitation of plant productivity under chronic N deposition. Root- and hyphal traits associated with nutrient-mining strategy including root- and hypha-derived C inputs, root effect and hypha effect on activities of N- and P-acquisition enzymes in the rhizosphere and hyphosphere. Root- and hyphal traits associated with nutrient-foraging strategy including root biomass, root diameter, specific root length, hyphal biomass, and hyphal exploration types. [Colour figure can be viewed at wileyonlinelibrary.com]

deposition. This highlights that ECM fungal hyphae and associated hyphosphere effect play an essential role in belowground nutrient acquisition and plant adaption to changing environments in ECM-dominated forests.

4.1 | Consistent pattern of trade-offs between root nutrient-mining and nutrient-foraging strategies with N addition

The PCA analysis showed that root functional traits were separated by N-addition conditions along the PC1 axis in both forests, shifting from nutrient mining in the control plots toward nutrient foraging in the N-addition plots (Figure 5a,b) which supports our first hypothesis. There were three pieces of evidence for the trade-off between root nutrient-mining and nutrient-foraging strategies. First, N addition decreased root-derived C input in both forests, consistent with previous studies reporting decreased belowground C allocation under N addition in forests based on the negative responses of root biomass, root exudation, and root respiration (Fransson & Johansson, 2010; Janssens et al., 2010; Nadelhoffer, 2000). This suggests that the N-induced increase in available N supply reduced belowground C investment to roots. Second, the activity of rhizosphere microbes to mineralize organic N might be inhibited due to the increased microbial C limitation induced by reductions in root-derived

C in both forests. Under N-poor conditions, roots often interact with soil microbes for N acquisition by providing large amounts of labile C (Brzostek et al., 2013; Dijkstra et al., 2013). However, when soil N availability is increased, the interactions between roots and soil microbes are decoupled (Carrara et al., 2018, 2022). Thus, the RE on N-acquisition enzyme activity decreased along with decreasing root-derived C input under N addition (Figure 2b). Third, increased SRL and root-branching numbers and decreased root diameter indicated that fine roots in both forests became longer and thinner with a more complex architecture under N addition. This is considered an efficient morphological strategy of ECM roots to enhance nutrient foraging (Chen et al., 2016; Ostonen et al., 2017). Collectively, while reducing C allocation to rhizosphere organic N mining under enhanced N availability, ECM roots changed morphological traits to sustain N uptake, regardless of initial forest nutrient status.

4.2 | Varied patterns of trade-offs between hyphal nutrient-mining and nutrient-foraging strategies with N addition

Supporting our second hypothesis, the trade-off between hyphal nutrient-mining and nutrient-foraging strategies showed variable responses under N addition, as indicated by the different dominance of hyphal functional traits between the control and N-addition plots

in both forests (Figure 5c,d). We surmise that such variation under N addition between two forest is determined by variation in the ecosystem N/P limitation status and the nutrient demand of host trees and ECM fungi. For the *Pinus armandii* forest with relatively low N status, the added N is used to meet the demand of ECM fungi, which act as an "N-trap" (i.e., N immobilization in ECM hyphae) (Franklin et al., 2014; Högberg et al., 2021). The proposed "N-trap" effect is supported by the increased hyphal biomass (52% increase) and hyphal N concentration (145% increase) but reduced hyphal C:N ratio (58% decrease) and δ^{15}N signature (48% decrease) in the N-addition plots compared with the controls ($p < .05$, Table S3). Moreover, the greater abundance of C-S explorers of ECM fungi in the N-addition plots (Figure 4a,b) is in line with previous studies in which increased N availability favored N-tolerant and nitrophilic ECM fungal taxa with high hyphal N immobilization and short-distance exploration hyphae (Lilleskov et al., 2011, 2019; Suz et al., 2021). Therefore, due to the greater N-trap effect of ECM fungi under N addition, only small amounts of additional N were transferred to trees (Hasselquist & Högberg, 2014; Näsholm et al., 2013). Hence, trees still tended to allocate more C to support ECM fungi and produce extraradical hyphae to cope with N limitation, as indicated by the increased hypha-derived C input associated with a greater HE on N-acquisition enzyme activity in the N-addition plots (Figure 2d,e).

In contrast to the *Pinus armandii* forest, the dominant hyphal functional traits under N addition in the *Picea asperata* forest with relatively high N status pointed to enhanced abilities of organic P mineralization and P foraging. ECM fungi enhance plant P acquisition via different mechanisms such as increasing long-distance explorers and enhancing mobilization of soil-bound inorganic or organic P by releasing exudates (e.g., organic acids) or phosphatases (Agerer, 2001; Köhler et al., 2018; Tedersoo & Bahram, 2019). Based on the present results, we assume that ECM fungi might exhibit two strategies to mitigate N-induced P limitation. One strategy is to enhance the production of P-acquisition enzymes from hyphae and/or hyphosphere microbes which is supported by the increased HE on P-acquisition enzyme activity under N addition in the *Picea asperata* forest (Figure 2f). Alternatively, N addition increased the abundance of M-L explorers (e.g., *Amphinema*) while decreasing C-S explorers under N addition in the *Picea asperata* forest (Figure 4c,d; Guo et al., 2021). This indicates that ECM fungi change their community structure to efficiently acquire P, since the medium-distance smooth exploration and long-distance exploration taxa are considered to have greater hyphal extensibility and enzymatic capabilities than the contact- and short-distance exploration taxa (Lilleskov et al., 2011). Some previous studies also showed that specific ECM fungal species with rhizomorphic long-distance exploration types were stimulated (e.g., *Paxillus involutus*, *Tylopilus*, and *Imleria badia*), and are good candidates for effective P foraging under high-N conditions (Almeida et al., 2019; Lilleskov et al., 2011). In addition to the hyphosphere priming effect, ECM fungi can also stimulate SOM decomposition by direct enzymatic breakdown and oxidation via Fenton chemistry (Frey, 2019; Miyauchi et al., 2020; Shah et al., 2016). In this study, the relative abundance of some ECM fungal taxa involved in enzymatic breakdown and Fenton chemistry (e.g., *Russula*, *Tuber* and *Piloderna*) were increased by N addition in the *Pinus armandii* forest (Figure 4a; Figure S1). In contrast, in the *Picea asperata* forest, ECM fungal taxa, such as *Trichphaea*, *Cortinarius*, and *Amanita*, were decreased or absent under N addition (Figure 4c; Figure S1). All these changes in ECM fungal community composition would also affect the release of N from SOM. Therefore, the intensity of a hyphosphere priming effect on nutrient-mining and the transition of ECM fungal community are good predictors and adaptive strategies for forest nutrient limitation. Overall, the changes in ECM fungal functional traits under N addition in two forests with contrasting initial nutrient status suggest that the trade-off of hyphal nutrient-acquisition strategies would shift from enhanced N acquisition toward enhanced P acquisition with the change in nutrient limitation of forest productivity from N to P limitation in the context of increased N deposition.

4.3 | Implications for plant–microbe–soil interactions under elevated N deposition

In conclusion, our study demonstrates that ECM extraradical hyphae exhibit more plastic nutrient-acquisition strategies than roots do under N deposition. Considering the increasing evidence that mycorrhizal association represents a "trait integrator" of functional traits to affect plant–plant interactions and ecosystem processes (Chen et al., 2016; Orwin et al., 2011; Rosling et al., 2015; Tedersoo et al., 2020), we propose a conceptual framework to appeal to more studies on trade-offs between root and hyphal nutrient-acquisition strategies across a wide range of forests shifting from N- to P-limitation under chronic N deposition (Figure 6). In terms of root nutrient-acquisition strategies, roots become thinner, highly extended, and form a more complex branching structure and invest less C in priming N-mining under enhanced N supply, suggesting that ECM roots shift their N-acquisition strategy from mining of organic N toward foraging for inorganic N. In contrast, the hyphal nutrient-acquisition strategies shifted from N acquisition toward P acquisition depending on forest nutrient limitation status and the nutrient demand of host trees and ECM fungi under chronic N addition. More specifically, in the N-poor forests (e.g., the *Pinus armandii* forest in this study), short-term or low-level N deposition primarily alleviated the N limitation of ECM fungi (e.g., increased ECM hyphal biomass and N concentration) rather than that of the host trees. Host trees increased C allocation to hyphae that enhanced organic N mineralization (i.e., enhanced hypha-derived C input and associated increase in a RE on N-acquisition enzyme activity). Another scenario is that the accumulation of N is sufficient to trigger ecosystem P limitation. For example, in the *Picea asperata* forest in this study, hyphal functional traits were affected to enhance P acquisition by inducing greater HE on phosphatase activity for organic P mineralization and modifying ECM fungal hyphal exploration types (i.e., increased abundance of medium- to -long-distance explorers and reduced abundance of contact-short distance explorers) to increases P foraging (Figure 6).

We suggest that extrapolating our conclusions to other forest ecosystems should be done with caution, given some potential caveats associated with the experimental system in the present study (e.g., relatively short duration, single level of N addition and only two forest types). Nevertheless, the present results highlight the importance of identifying differentiated responses of root and hyphal nutrient-mining- and nutrient-foraging traits to increased N addition. More long-term network studies are allowed to illuminate the underlying mechanism of tree adaption and forest function stability in ECM-dominated forests under changing environments.

AUTHOR CONTRIBUTIONS

Xiaomin Zhu, Huajun Yin, and Ziliang Zhang conceived and supervised the study; Xiaomin Zhu, W. Guo, and Huajun Yin performed the experiment and collected the data; Xiaomin Zhu and Dongdong Chen analyzed the data and drew the figures. Xiaomin Zhu, Ziliang Zhang, and Huajun Yin discussed the data and wrote the manuscript. Zhanfeng Liu and Hans Lambers edited the final draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c59zw3rdj

ORCID

Xiaomin Zhu https://orcid.org/0000-0001-8213-1992
Zhanfeng Liu https://orcid.org/0000-0002-6602-6196
Huajun Yin https://orcid.org/0000-0001-9202-8286

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SUPPORTING INFORMATION

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