NITROGEN ENRICHMENT RESTRUCTURING ROOT-FUNGUS COORDINATION THROUGH TRAIT-BASED PARTITIONING AND SYMBIOSIS-DEPENDENT FEEDBACK DECOUPLING

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Abstract. Atmospheric nitrogen (N) deposition is intensifying, yet its effects on fine-root and mycorrhizal trait coordination across mycorrhizal types and root functions remain poorly understood, limiting predictions of belowground responses. We conducted a factorial field experiment across 12 plots in a temperate forest, using five dominant tree species (three Ectomycorrhizal (ECM), two Arbuscular mycorrhizal (AM)), four N treatments (0, 25, 50, 75 kg $N \cdot ha^{-1} \cdot yr^{-1}$), and functional root classifications (absorptive vs. transport roots), to evaluate integrated shifts in morphological, chemical, and mycorrhizal traits. N enrichment significantly altered root traits in a symbiosis- and function-dependent manner. ECM transport roots showed 38–61% increases in diameter and length under 50–75 kg $N \cdot ha^{-1} \cdot yr^{-1}$, whereas AM absorptive roots increased specific root length (SRL) (8.3–10.1 $m \cdot g^{-1}$) and surface area (6.9–9.5 $cm^2 \cdot g^{-1}$) at moderate N levels. Root tissue density (RTD) declined by 22–45% across both types. Mycorrhizal root colonization (MRC) and hyphal traits decreased by 58% in AM roots but remained stable in ECM roots. Root N concentration increased by 19–35%, while P content declined in AM absorptive roots. Our results reveal a N-driven reorganization of root–fungus coordination, governed by symbiotic identity and root function. The observed trait plasticity and nutrient decoupling underscore a shift toward plant-dominant nutrient uptake strategies, highlighting the vulnerability of soil-plant-fungus feedback loops under chronic N loading. These findings provide critical trait-based evidence for forecasting nitrogen-driven shifts in ecosystem nutrient cycling and root-fungus functional decoupling, while offering mechanistic insight relevant to soil stability and ecological slope-engineering practices under intensified nutrient loading.
Keywords: *N addition, root morphological traits, root chemical traits, mycorrhizal types, mycorrhizal morphological characteristics*

Introduction

The structure and function of fine roots and their mycorrhizal symbionts constitute a fundamental component of forest nutrient cycling, mediating water acquisition, nutrient assimilation, and belowground carbon allocation (Saha et al., 2023; Ma et al., 2021). These traits collectively regulate plant-soil feedbacks, ultimately shaping ecosystem stability (Sveen et al., 2024). Among belowground symbioses, ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) associations represent two dominant nutrient acquisition strategies, differing in their resource foraging pathways and carbon costs (Yang et al., 2022; Stuart et al., 2020). However, this finely balanced system is increasingly challenged by sustained external nitrogen loading, which induces long-term

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nutrient stoichiometric imbalance, modifies soil chemistry, and imposes persistent pressure on plant-fungal coordination, thereby shifting allocation strategies within the soil-plant continuum (Viancelli et al., 2024; Qiu et al., 2023). In temperate forests of Northeast China, elevated N inputs (~18.7 kg N ha^{-1} yr^{-1}) interact with acidic soils and uneven nutrient distribution, generating uncertain feedbacks in root-fungus coordination (Hou et al., 2024). These complex responses underscore an urgent need to understand how exogenous N shapes root traits and mycorrhizal functioning under varying symbiotic types (Genre et al., 2020; Zhang et al., 2024). Yet, our mechanistic understanding of such responses remains limited, particularly regarding mycorrhizal-mediated root plasticity and nutrient partitioning under N enrichment (Tan et al., 2025; Schreider et al., 2022).

Several critical knowledge gaps restrict current insights into N-root-fungus interactions. First, most studies have examined N effects on root morphology in isolation, without accounting for the modifying role of mycorrhizal types or their influence on nutrient allocation strategies (Ma et al., 2023). This limitation hampers our understanding of how N alters the trait-based coordination between roots and fungi. Second, existing research rarely integrates both morphological and chemical root traits with corresponding mycorrhizal traits, restricting our ability to assess symbiotic responses at the whole-system level (Freschet et al., 2021). Third, the differential responses of ECM and AM fungi to N enrichment—particularly in terms of carbon trade-offs and nutrient foraging strategies—remain poorly resolved (Zhu et al., 2023). This restricts our understanding of how nutrient stoichiometry and fungal metabolism jointly determine root-fungus coupling under N stress. Collectively, these gaps limit predictive capacity regarding how temperate forest systems respond to sustained N deposition.

To address these gaps, we implemented a field-based N addition experiment in a temperate mixed forest of Northeast China, targeting five ecologically dominant tree species that span divergent mycorrhizal strategies. Specifically, we selected three ECM-associated species (*Pinus koraiensis*, *Abies nephrolepis*, *Tilia amurensis*) and two AM-associated species (*Acer tegmentosum*, *Fraxinus mandshurica*), chosen for their contrasting root morphologies, symbiotic affiliations, and nutrient foraging traits. These species represent key functional types in mature broadleaf-Korean pine forests, enabling direct comparison of ECM and AM responses under controlled N enrichment. By integrating species identity, root functional class (absorptive vs. transport roots), and mycorrhizal morphological traits, our study provides a mechanistic framework to examine how N enrichment restructures root-fungus coordination. This experimental system offers a unique opportunity to evaluate trait-based feedbacks under chronic N deposition, thereby contributing novel insights into symbiosis-mediated belowground adaptation.

Building on the contrasting ecological strategies of ECM and AM associations, we hypothesize that (*H1*) N enrichment differentially modulates fine-root morphological traits through a trait-based partitioning mechanism governed by mycorrhizal type and root functional class. Specifically, N availability is expected to interact with symbiotic affiliation and root order to shape structural plasticity in absorptive versus transport roots (Yang et al., 2025). ECM-associated roots may exhibit increased diameter and tissue density to support structural reinforcement and organic nutrient acquisition, while AM-associated roots are hypothesized to allocate biomass toward elongation and surface expansion, facilitating efficient exploitation of inorganic N pools (Guo et al., 2021; Weigelt et al., 2021). These responses reflect a coordinated adjustment in root construction cost and foraging precision, indicating that functional differentiation among

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root classes amplifies symbiosis-specific adaptive strategies (Kuyper et al., 2021). By integrating the dimensions of N supply, mycorrhizal identity, and root topology, this hypothesis addresses how nutrient deposition may restructure belowground resource acquisition patterns across forest tree species, ultimately influencing nutrient turnover and ecological competitiveness under global change scenarios.

We further hypothesize that (*H2*) N enrichment suppresses mycorrhizal morphological development and decouples soil-plant-fungus feedbacks in a symbiosis-type-dependent manner. As elevated N reduces host reliance on fungal foraging, it may limit carbon allocation to mycorrhizal partners, thereby diminishing hyphal proliferation and colonization intensity—particularly in AM associations, which lack external mantles and rely on rapid, carbon-sensitive signaling (Bell et al., 2024). ECM fungi, in contrast, are expected to maintain greater morphological integrity due to their reliance on more recalcitrant nutrient sources and thicker sheathing structures (Coleine et al., 2022). Simultaneously, root N (RN) and phosphorus (RP) concentrations are anticipated to shift in response to these altered symbiotic dynamics, with ECM roots accumulating more nutrients owing to enzymatic capacity and sustained fungal interface. These changes suggest that N-driven breakdown of cooperative nutrient exchange pathways may reshape root nutrient profiles, leading to functional decoupling between root chemical composition and mycorrhizal morphology (Zhou et al., 2025). This hypothesis provides a mechanistic foundation for understanding how atmospheric N deposition alters belowground mutualisms and nutrient flows, with implications for long-term ecosystem functioning and resilience.

Materials and methods

Study sites and experiment design

Our experimental site is located in the core transition zone of the Xiaoxing'an Mountains-Songnen Plain ecoregion (48°05'–48°10'N, 129°02'–129°12'E), within Wuying National Nature Reserve (*Fig. 1*). The site covers 2400 ha and spans an elevation range of 280–520 m above sea level, forming a complex topographic mosaic shaped by Quaternary glacial processes. This glacial legacy produced alternating moridine ridges and lacustrine depressions, resulting in pronounced microtopographic heterogeneity with up to 22% slope variation. The temperate continental monsoon climate drives strong seasonality ($\Delta T_a nn = 42.5°C$, MAP = 630 mm), with 78% of annual precipitation occuring from June to August, interacting with Haplic Luvisols (dark brown forest soils) whose baseline properties were assessed before treatment initiation (C/N = 16.3 ± 2.1; bulk density = 1.28 ± 0.15 g/cm³), indicating moderate N availability (Jiao et al., 2021). This edaphic-climatic template supports a stratified forest ecosystem characterized by: A semi-open canopy layer (25–32 m height, LAI = 5.7) dominated by *P. koraiensis* (IV = 28.6%) and *A. nephrolepis* (IV = 19.3%), A subcanopy (8–15 m) co-dominated by *A. tegmentosum* and *T. amurensis*, and An understory hosting 36 shrub species (e.g., *Corylus mandshurica*, constancy = 45%) and 23 herbaceous taxa with *Raunkiaer* life-form dominance (34% hemicryptophytes) (Zhenet al., 2024).

Our stratified sampling design targeted five foundation tree species (*P. koraiensis, A. nephrolepis, A. tegmentosum, T. amurensis, F. mandshurica*; 73% total basal area) across aspect-controlled transects spanning NE-facing *F. mandshurica* slopes to SW-facing *Betula platyphylla* communities. These species were selected based on their dominance in local forest composition and their distinct mycorrhizal affiliations (three ECM-

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associated and two AM-associated), which provide a representative model system to examine symbiosis-specific nutrient strategies under N enrichment (*Table 1*). This configuration captures ecotonal transitions at 430 m asl, where soil-water holding capacity (38–42% v/v) and coarse woody debris (12.3 Mg/ha) underpin a carbon sequestration potential of 6.2 ± 1.3 tC/ha/yr, quantified via eddy covariance towers established in 2024 (Yan et al., 2021).

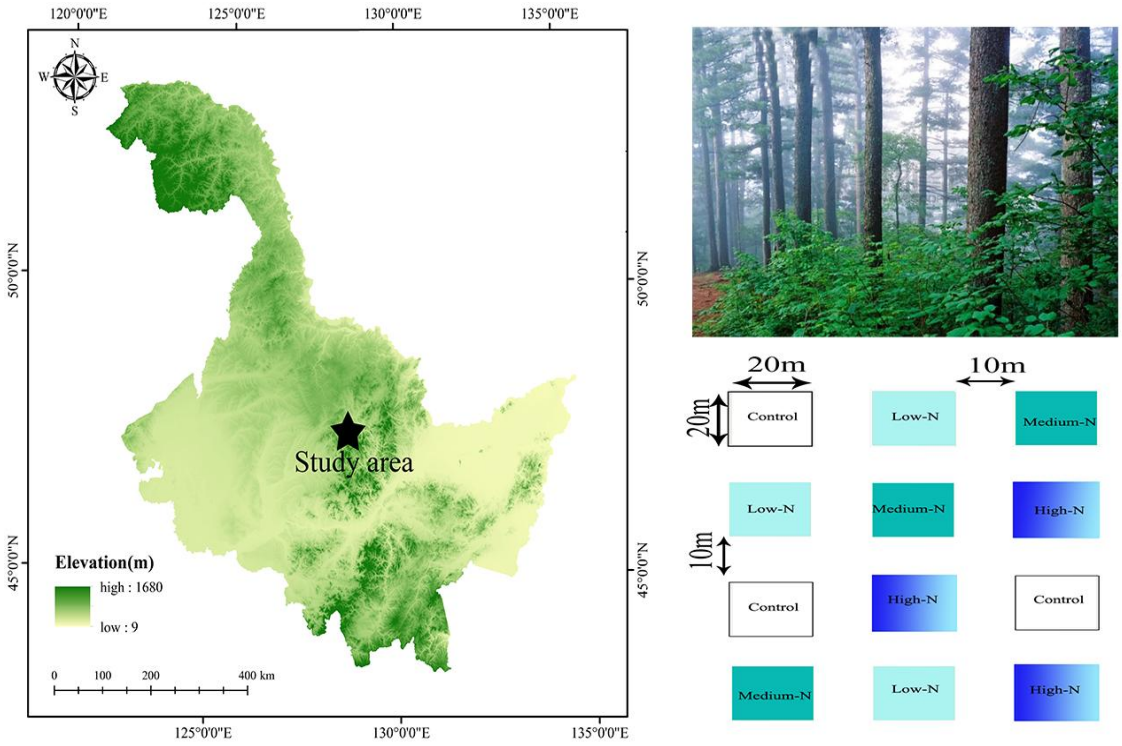

Figure 1. *Sample plot and diagram of the experimental design in Wuying national nature reserve, Yichun city, Heilongjiang province, northeastern China*

Table 1. *Ecological characteristics of the 5 species studied*

Family	Species	Abbreviation	Mycorrhizal
Pinaceae	*Pinus koraiensis* Siebold & Zucc.	*P. koraiensis*	ECM
Pinaceae	*Abies nephrolepis* (Trautv.) Maxim.	*A. nephrolepis*	ECM
Malvaceae	*Tilia amurensis* Rupr.	*T. amurensis*	ECM
Sapindaceae	*Acer tegmentosum* Maxim.	*A. tegmentosum*	AM
Oleaceae	*Fraxinus mandshurica* Rupr.	*F. mandshurica*	AM

Situated within the Wuying National Nature Reserve's eastern montane ecotone, our replicated experimental system comprises twelve 400-m² (20 × 20 m) plots established using a randomized complete block design (RCBD). The plots were strategically positioned across 15°–28° slopes with azimuth-controlled placement to capture vegetation-habitat gradients (mean coverage index = 0.72 ± 0.08). Plots were isolated through 10-m native litter buffer zones and aspect-aligned drainage channels, effectively suppressing N cross-contamination ($\delta 15^5$N tracer verification <2.3% lateral transfer) while

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maintaining natural microclimate continuity (≥80% canopy preservation). Four N regimes (0/25/50/75 kg $N \cdot ha^{-1} \cdot yr^{-1}$ NH_4NO_3) were administered monthly via pneumatic atomization during stomatal closure (05:30–06:30 local time) from May to October in 2011–2024, synchronized with growing degree days (Guan et al., 2023). Sampling for the present study was performed in July 2024, after 13 growing seasons of treatment.

The experiment included four N addition treatments: control (0 kg $N \cdot ha^{-1} \cdot yr^{-1}$), low-N (25 kg $N \cdot ha^{-1} \cdot yr^{-1}$), medium-N (50 kg $N \cdot ha^{-1} \cdot yr^{-1}$), and high-N (75 kg $N \cdot ha^{-1} \cdot yr^{-1}$). These treatments were applied across 12 experimental plots. Additionally, five tree species were studied: *P. koraiensis, A. nephrolepis, A. tegmentosum, T. amurensis, and F. mandshurica*. The occurrence and relative abundance of these species within each plot reflected the natural species composition, and no systematic differences in species distribution were observed among plots before nitrogen addition. These tree species were further categorized based on their mycorrhizal type, with three species *(P. koraiensis, A. nephrolepis, T. amurensis*) classified as ECM (ectomycorrhizal) and two species (*A. tegmentosum, F. mandshurica*) classified as AM (arbuscular mycorrhizal). Therefore, the study involved four experimental factors: N treatment (4 levels), tree species (5 species), mycorrhizal type (2 types: ECM and AM), and root functional class (absorptive vs. transport roots).

Sample collection

We used a randomized block design with 12 plots (3 blocks × 4 N levels). The plot was treated as the experimental unit. For each species within a plot, we sampled three trees (nested within plot) and composited measurements at the tree or root level as specified below. Statistical models accounted for the hierarchical structure via random effects.

Root samples were collected within a 3-meter radius around each selected tree using a soil auger with a diameter of 10 cm and a depth of 10 cm. This sampling depth was chosen based on prior findings that over 70% of roots are distributed within the top 10 cm of soil, corresponding to the A horizon as classified by the USDA Soil Taxonomy (Kyebogola et al., 2020).

Each soil core was carefully loosened by hand to extract intact root segments, encompassing root orders 1 through 5. Root samples from three soil cores collected around each tree were combined to form a composite sample, ensuring a representative root sample for each tree. All root samples were immediately placed in ice-cooled storage bags and transported to the laboratory within 3 h. Upon arrival, the samples were stored at −20°C to preserve root integrity for subsequent analyses of morphological and chemical traits (*Table 2*).

In addition to root sampling, soil from each extracted core was collected and placed into ice-cooled storage bags. These soil samples were transported to the laboratory under the same conditions and stored at −20°C for subsequent analyses. The soil samples were used to measure mycorrhizal characteristics (*Table 2*). In the laboratory, root samples were thawed and gently washed with deionized water to remove residual soil. Cleaned roots were sorted by species and root functional class and then scanned using WinRHIZO Pro (Regent Instruments Inc., Canada) to obtain measurements of root diameter, length, surface area, and volume. Specific root length (SRL, m g^{-1}) was calculated as total root length divided by root dry mass, and root tissue density (RTD, g cm^{-3}) was calculated as root dry mass divided by root volume. After scanning, roots were oven-dried at 65°C for 72 h and weighed for dry biomass determination.

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Table 2. *Symbols*

Traits	Abbreviation	Units	Description
Root morphological traits			
Mean root diameter	RD	mm	Averaged mean root diameter of all the nine spots of concerned roots
Mean root length	MRL	mm	Length per unit number of roots
Specific root length	SRL	$m \cdot g^{-1}$	Length per unit root dry mass
Mean root surface area	RSA	$cm^2 \cdot g^{-1}$	Surface area per dry mass
Root tissue density	RTD	$g \cdot cm^{-3}$	Mass per unit root volume
Root chemical traits			
Root N concentration	RN	$g \cdot kg^{-1}$	Total N content per unit root dry mass
Root P concentration	RP	$g \cdot kg^{-1}$	Total phosphorus content per unit root dry mass
Mycorrhizal morphological characteristics			
Total hyphae length	THL	$cm \cdot g^{-1}$	Total length of hyphae measured within the sampled area
Total hyphae surface area	THS	$cm^2 \cdot g^{-1}$	Total surface area of hyphae measured within the sampled area
Mean hyphae diameter	MHD	mm	Averaged diameter of hyphae measured within the sampled area
Mycorrhizal root colonization	MRC	%	Percentage of root colonized by mycorrhizal fungi

Morphological analysis

Following field collection, root systems underwent rigorous laboratory processing to ensure biomechanical integrity and analytical precision. Samples were aseptically rinsed in three cycles of deionized water with fine-tipped forceps until achieving optical transparency under 10× magnification (Leica S9i, Leica Microsystems, Germany), eliminating all rhizosphere particulates. The viability of roots was determined based on characteristics such as color, elasticity, structure, and texture. Each root sample was divided into two portions. One portion was reserved for mycorrhizal infection rate determination, and the other was used for root morphological analysis.

For root samples designated for morphological analysis, classification was performed based on root topological structure, where the terminal position was defined as first-order roots, the junction of two first-order roots as second-order roots, and so forth, resulting in a detailed classification into five root orders (1–5). To enable a comparative analysis of resource acquisition and translocation strategies, root orders were categorized following hierarchical topological classification, functional trait continuum analysis, and the root economic spectrum theory (Yu et al., 2025). A preliminary anatomical experiment was conducted on root orders 1–5 during sample preparation. Anatomical sections revealed that the cortical proportion of first- to third-order roots reached 65–78%, whereas the xylem vessel area ratio of fourth- to fifth-order roots increased to 42–55%, aligning with the functional differentiation theory proposed by Li et al. (2022). Therefore, roots of orders 1–3 were classified as absorptive roots, while roots of orders 4–5 were categorized as transport roots.

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An anatomical pre-experiment was conducted. Key structural traits, including: cortical thickness, stele diameter, xylem development, and Casparian strip presence, were analyzed. Absorptive roots were expected to exhibit larger cortical regions, while transport roots were characterized by thicker steles and well-developed xylem.

The roots of each tree species were scanned using an Epson 11000XL scanner (produced by Seiko Epson Corporation, Japan) at a resolution of 600dpi. Root morphological parameters including root number, average root diameter, individual root length, total root length, individual root surface area, and root volume were measured using root analysis software (produced by Regent Instruments Inc., Quebec, Canada) (Tanikawa et al., 2021). The root samples were dried in an oven at 75°C until a constant weight was achieved. Subsequently, the samples were weighed. The specific root length (SRL, in cm/g) was calculated as the total root length divided by the corresponding dry mass. Root tissue density (in g/cm3) was determined by dividing the dry weight by the corresponding total volume.

Extraradical hyphal network isolation and morphometric profiling

In each experimental plot, primary lateral root systems of target tree species were systematically sampled following a randomized quadrant collection protocol. For each biological replicate, 200–300 intact root tips (diameter range: 0.5–1.2 mm) were aseptically excised using precision scissors, ensuring minimal mechanical damage to cortical tissues. For ECM roots, initial symbiotic assessment was based on morphological adaptations (e.g., root tip hypertrophy, mantle formation) observed under a stereomicroscope (Leica M205C, 10–40×). For AM roots, internal colonization structures were not visible without staining; therefore, trypan blue staining was conducted prior to microscopic evaluation (Moukarzel et al., 2020). For AM roots, we employed trypan blue staining (0.05% in lactoglycerol) following root clearing with 10% KOH, and estimated mycorrhizal colonization rates using the magnified intersection method under 200× magnification. For ECM roots, colonization was assessed based on morphological criteria (mantle formation, dichotomous branching) using a stereomicroscope. The percentage of colonized root tips was calculated as (number of colonized tips × 100)/total number of tips.

For extraradical hyphal extraction, soil samples (5 g fresh weight from the rhizosphere zone) underwent wet-sieving and filtration procedures modified from standard protocols (Boyno et al., 2023). We acknowledge that this approach may include hyphae from non-mycorrhizal fungi (e.g., saprotrophs, pathogens); thus, our measurements reflect total soil fungal hyphae rather than strictly mycorrhizal hyphae. To minimize this limitation, we selected sampling zones closely associated with live roots and used hyphal morphology (diameter, septation, structure) and spatial context as auxiliary criteria during microscopy. Specifically, soil suspensions were mechanically homogenized using a calibrated laboratory blender (DW-3A-90W, 15,000 rpm for 30 s, produced by Shanghai Anting Scientific Instrument Factory, China) in 500 mL deionized water, followed by sequential filtration through 100-μm and 40-μm mesh sieves to remove particulate debris. The resultant hyphal suspension was vacuum-filtered onto polycarbonate membranes (24 mm diameter, 0.4 μm pore size; Whatman Nuclepore) using a standardized aspiration protocol (1 mL aliquot per membrane). Membranes were subsequently mounted on glass slides with lactoglycerol mounting medium for brightfield microscopy analysis (Nikon Eclipse Ni-U, 400× magnification, produced by Nikon Corporation, Japan), with hyphal morphological characteristics documented through systematic grid scanning (20 fields per membrane).

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After filtration, a drop of lactoglycerol solution containing 0.05% trypan blue was added onto the filter membrane to stain the hyphae. The hyphae on the filter membrane were magnified and photographed using a compound microscope (model: BX-51, produced by Olympus Corporation, Japan). Using root analysis software, the total length, total surface area, and average diameter of the hyphae were measured. Each soil sample was subjected to the above operations three times to obtain the average values of hyphal characteristics.

Statistical analysis

The normality of all variables, including root morphological traits (RD, SRL, MRL, RTD, RSA), root chemical traits (RN and RP), and mycorrhizal morphological traits (THL, THS, MHD, MRC), was tested using Shapiro-Wilk tests. Homogeneity of variances was assessed using Levene's tests. Data transformations were applied as needed to meet the assumptions of normality and homogeneity before further analyses. A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of N addition treatments (CK, TL, TM, TH), mycorrhizal types (ECM and AM), and their interactions on root morphological and chemical traits. Tukey's post hoc tests were applied to identify significant differences among treatments. For mycorrhizal morphological traits, the interaction effects of N addition treatments, species, and mycorrhizal types were assessed using a three-way ANOVA. To further analyze the effects of N addition levels, tree species, and root types (transport and absorptive roots) on fine-root traits, a linear mixed-effects model was employed. Fixed effects included N addition treatments, mycorrhizal types, species, root types and their interactions, while random effects accounted for interspecific variability. The same model structure was used to determine the effects of treatments, species, and mycorrhizal types on mycorrhizal morphological traits. All statistical analyses were performed using R (version 4.3.1; R Core Team, 2023).

Results

Fine root morphological traits

N treatments significantly affected fine-root morphological traits, with clear differentiation across mycorrhizal types and root classes. In transport roots of ECM species, mean root diameter was significantly higher under the TM and TH treatments compared to CK and TL (*Fig. 2A*), and mean root length was significantly greater under TH than under other treatments (*Fig. 2B*). In absorptive roots of AM species, specific root length was significantly higher under MN than under CK and TH (*Fig. 2C*), and root surface area was significantly higher under TM and TH than under CK and TL (*Fig. 2D*). Root tissue density was significantly lower under all N addition treatments compared to CK in both ECM and AM roots, particularly in transport roots (*Fig. 2E*). According to the ANOVA results, N treatment, mycorrhizal type, and root type each had significant main effects on mean root diameter, specific root length, mean root length, and root tissue density (*Table 3*). Significant two-way (M × T, M × R, T × R) and three-way (M × T × R) interactions were also detected for these traits (*Table 3*), indicating that fine-root morphological responses were significantly shaped by the combined effects of N availability, symbiotic association, and functional root classification.

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Table 3. *Analysis of variance results for effects of mycorrhizal type (ECM and AM), treatments, root type (transport roots and absorptive roots) and interactions on fine root traits*

	P value							
	df	**RD**	**SRL**	**MRL**	**RTD**	**RSA**	**RN**	RP
M	1	0.028*	0.022*	<0.001**	0.049*	0.178	<0.001**	<0.001**
T	3	0.966	0.964	<0.001**	0.137	0.038*	<0.001**	0.142
R	1	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	0.221
M × T	3	0.896	0.041*	<0.001**	0.023*	0.672	<0.001**	0.225
M × R	1	<0.001**	<0.001**	<0.001**	0.018*	0.133	<0.001**	0.518
T × R	3	<0.001**	0.023*	<0.001**	0.632	0.022*	<0.001**	0.027*
M × T × R	3	<0.001**	<0.001**	<0.001**	<0.001**	0.128	<0.001**	0.712

*p < 0.05; ** p < 0.01. Shown are degrees of freedom (df) and the P value of the respective variables and the model itself. M represents the mycorrhizal type (ECM and AM); T represents the treatments (control, low-N (25 kg N·ha^{-1}·yr^{-1}), medium-N (50 kg N·ha^{-1}·yr^{-1}), high-N (75 kg N·ha^{-1}·yr^{-1})); R represents the root type (transport roots and absorptive roots). RD represents the averaged mean root diameter; SRL represents the specific root length; MRL represents the mean root length; RTD represents the root tissue density; RSA represents the mean root surface area; RN represents the root N concentration; RP represents the root P concentration

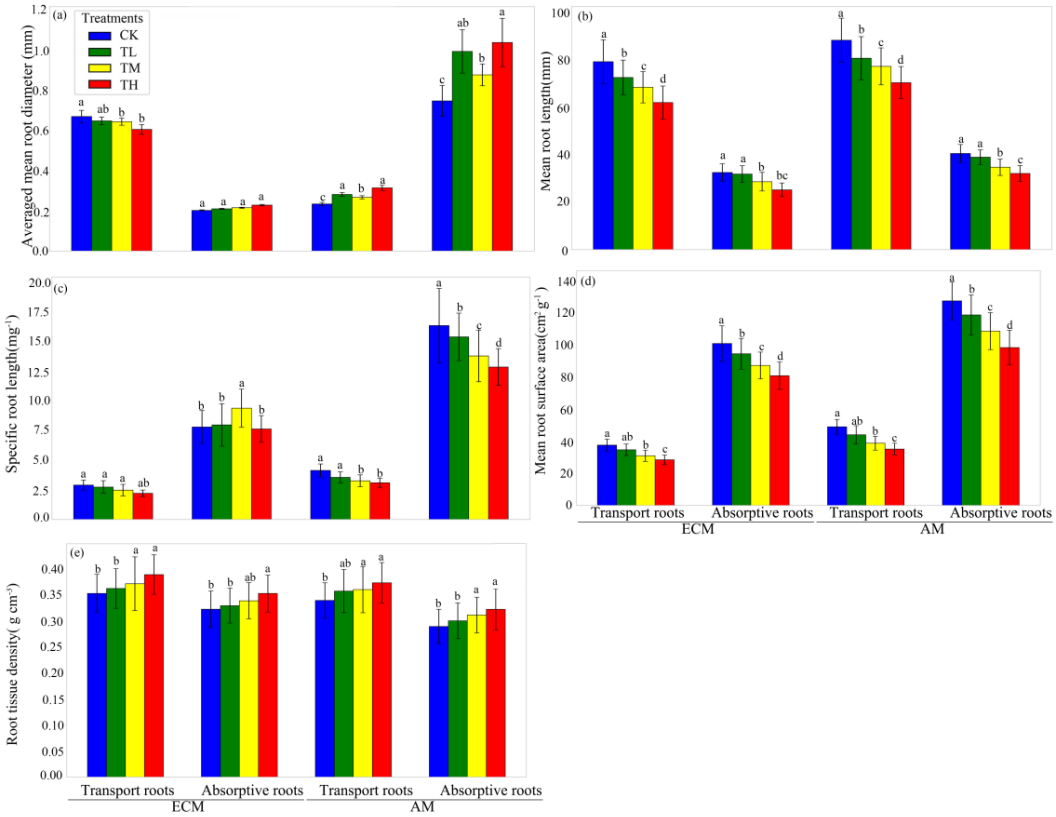

Figure 2. *Averaged mean root diameter (a), mean root length (b), specific root length (c), mean root surface area (d) and root tissue density (e) of different root type (transport roots and absorptive roots) under different treatments in different mycorrhizal types (ECM and AM) tree species. Error bars represent the standard error values of the numerical data. Significant differences (P < 0.05) were determined using Tukey's HSD post hoc tests and are indicated by different lower-case letters. CK represents the control, TL represents the low-N (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N (50 kg N·ha^{-1}·yr^{-1}), TH represents the high-N (75 kg N·ha^{-1}·yr^{-1})*

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Root chemical traits

Root nitrogen (N) and phosphorus (P) concentrations exhibited significant variations across N addition treatments, mycorrhizal types, and root functional classes (*Fig. 3*; *Table 3*). In absorptive roots of arbuscular mycorrhizal (AM) trees, root N concentration was significantly higher under high N (TH) treatment than under control (CK), low-N (TL), and medium-N (TM) treatments (*Fig. 3A*). In contrast, in ectomycorrhizal (ECM) trees, both transport and absorptive roots under TM and TH treatments had significantly higher N concentrations than those under CK (*Fig. 3A*). AM absorptive roots under CK had significantly higher values than under TM and TH treatments, while ECM absorptive roots under CK and TL were significantly higher than those under TM and TH (*Fig. 3B*). Significant interaction effects of treatment, mycorrhizal type, and root class were observed for root N concentration ($P < 0.001$), while root P concentration was significantly affected by mycorrhizal type ($P < 0.001$), treatment ($P = 0.038$), and the interaction between treatment and root type ($P = 0.027$) (*Table 3*).

Mycorrhizal morphological characteristics

N addition significantly reduced all measured mycorrhizal morphological characteristics across both mycorrhizal types, with the most pronounced declines observed under the high-N treatment (TH). Specifically, mycorrhizal root colonization (MRC), mean hyphae diameter (MHD), total hyphae length (THL), and total hyphae surface area (THS) were significantly lower under TH compared to CK, TL, and TM, with AM-associated roots exhibiting consistently lower values than ECM-associated roots (*Fig. 4a–d*). Analysis of variance revealed that mycorrhizal type, N treatment, and their interaction exerted significant effects on all traits ($P < 0.001$ for MRC, THL, and THS; $P < 0.05$ for MHD), indicating strong and consistent treatment responses across symbiotic categories (*Table 4*).

Table 4. *Analysis of variance results for effects of category, treatments and interactions on mycorrhizal morphological characteristics*

	df	P value			
		THL	THS	MHD	MRC
Category	1	< 0.001**	< 0.001**	0.021*	< 0.001**
Treatments	3	< 0.001**	< 0.001**	0.032*	< 0.001**
Category × treatments	3	< 0.001**	< 0.001**	0.017*	< 0.001**

* $p < 0.05$; ** $p < 0.01$. Shown are degrees of freedom (df) and the P value of the respective variables and the model itself. Category (EM versus AM); Treatments (control, low-N (25 kg $N \cdot ha^{-1} \cdot yr^{-1}$), medium-N (50 kg $N \cdot ha^{-1} \cdot yr^{-1}$), high-N (75 kg $N \cdot ha^{-1} \cdot yr^{-1}$)). THL represents the total hyphae length; THS represents the total hyphae surface area; MHD represents the mean hyphae diameter; MRC represents the mycorrhizal root colonization

Discussion

N enrichment restructures fine-root morphology via symbiosis-dependent trait plasticity and functional differentiation

N enrichment significantly altered fine-root morphology in a manner dependent on mycorrhizal type and root function, supporting our first hypothesis. ECM transport roots thickened and elongated under moderate to high N, while AM absorptive roots exhibited

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increased SRL and surface area under moderate N addition (*Fig. 2A–D*). These divergent patterns reflect contrasting strategies: ECM roots enhance persistence and resource acquisition through structural investment, whereas AM roots adopt acquisitive, high-turnover traits to exploit transient nutrient availability (Strock et al., 2020; Friedel and Smith, 2021).

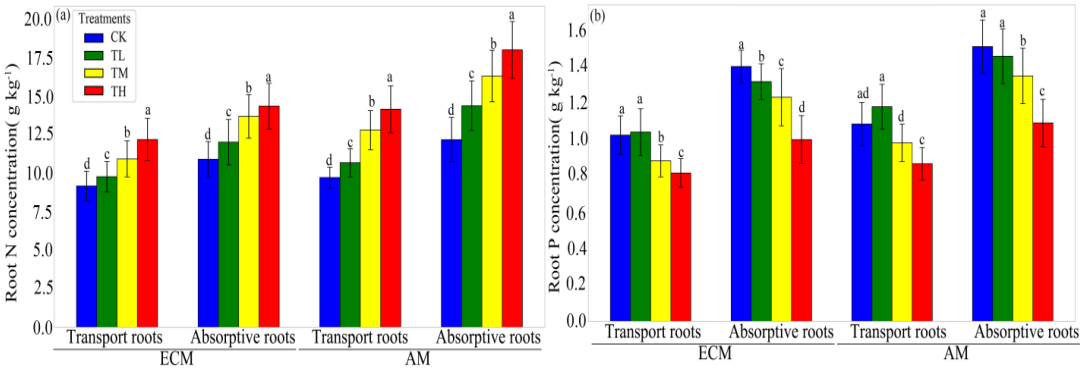

Figure 3. *Root N concentration (a) and root P concentration (b) of different root type (transport roots and absorptive roots) under different treatments in different mycorrhizal types (ECM and AM) tree species. Error bars represent 1SE of the mean. Significant differences ($P < 0.05$) between treatments are indicated by different lower-case letters. CK represents the control, TL represents the low-N (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N (50 kg N·ha^{-1}·yr^{-1}), TH represents the high-N (75 kg N·ha^{-1}·yr^{-1})*

The observed trait divergences underscore the role of symbiotic affiliation in shaping morphological plasticity, with ECM roots thickening and elongating potentially to maintain hydraulic conductivity and mechanical integrity under N-driven shifts in soil carbon dynamics (Zhao et al., 2022; Li et al., 2021). This aligns with the view that ECM fungi, reliant on enzymatic mineralization of organic substrates, may benefit from prolonged root residence and enhanced substrate contact (Tunlid et al., 2022). In contrast, AM absorptive roots exhibited increased SRL and surface area under moderate N availability, a trait syndrome associated with acquisitive foraging under rapid nutrient turnover (Isaac et al., 2021). These adjustments likely reflect the fungal strategies they support—AM fungi primarily absorb inorganic N forms from labile pools, requiring expansive but ephemeral fine-root networks (Dixon et al., 2020). Thus, root trait responses under N addition mirror the functional dichotomy between AM and ECM symbioses in nutrient foraging.

The structural shifts in RTD across all treatments provide further evidence for convergent reduction in construction costs under N enrichment (*Fig. 2E*), a trend consistent with global patterns of increased morphological plasticity and reduced structural persistence under nutrient-rich conditions (Rubio-Ríos et al., 2022). Contrary to studies reporting increased tissue density as a conservative response to nutrient abundance (Nair et al., 2020), the decrease observed here may reflect a shift toward short-lived, high-turnover strategies. This shift is likely reinforced by fungal-mediated carbon feedbacks, where elevated N availability reduces mycorrhizal carbon demand and thus weakens selection for robust, long-lived root structures (Wu et al., 2022). These interpretations are further supported by trait-longevity relationships observed across root economic spectra, whereby reduced RTD is consistently associated with shorter lifespan

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and faster turnover (Li et al., 2024). The strong interactions among N treatment, root class, and symbiotic type (*Table 3*) further indicate that morphological responses are filtered by both functional role and fungal affiliation, underscoring the need to assess root traits within integrated ecological contexts.

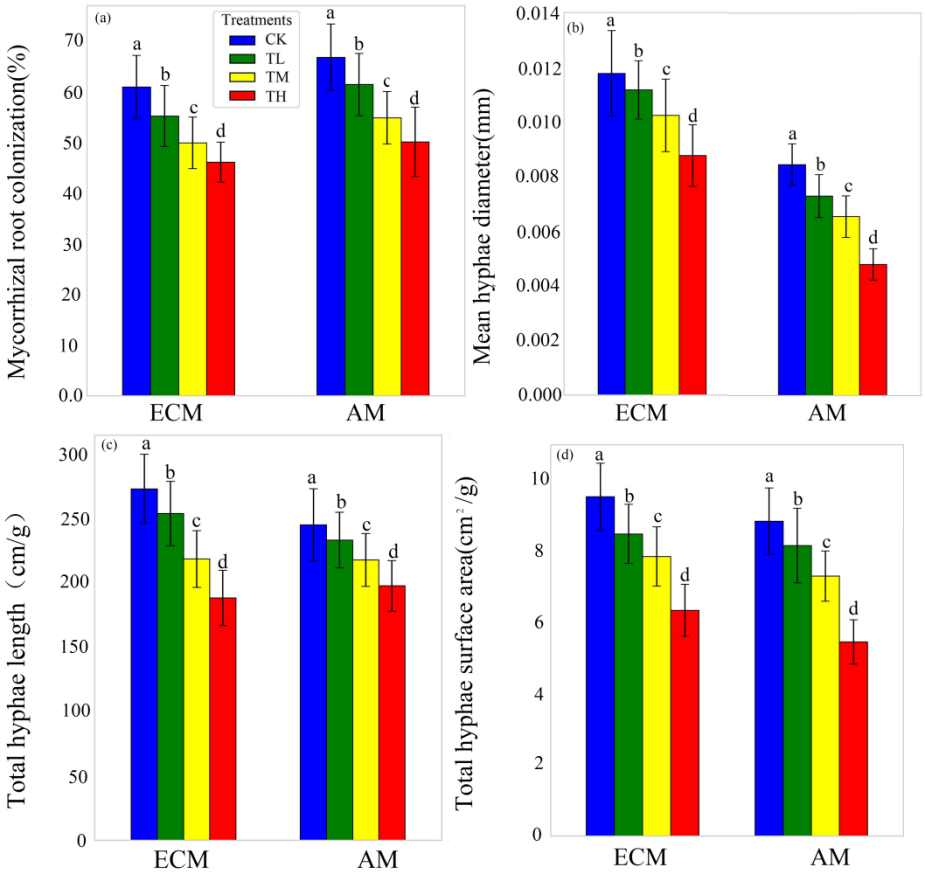

Figure 4. *Mycorrhizal root colonization (a), mean hyphae diameter (b), total hyphae length (c) and total hyphae surface area (d) of different treatments in different mycorrhizal types (ECM and AM) tree species. Error bars represent 1SE of the mean. Significant differences ($P < 0.05$) between treatments are indicated by different lower-case letters. CK represents the control, TL represents the low-N (25 kg N·ha^{-1}·yr^{-1}), TM represents the medium-N (50 kg N·ha^{-1}·yr^{-1}), TH represents the high-N (75 kg N·ha^{-1}·yr^{-1})*

N enrichment disrupts mycorrhizal morphology and decouples root-fungus nutrient feedbacks in a symbiosis-type-dependent manner

N-induced suppression of mycorrhizal morphological development and alteration of root nutrient profiles provide compelling evidence for the breakdown of cooperative nutrient exchange pathways under chronic deposition (Copeet al., 2022). The coordinated decline in mycorrhizal root colonization, hyphal length, and surface area under elevated N, particularly in AM symbioses, indicates that N enrichment triggers a shift in host-fungus interdependence, consistent with our second hypothesis (*Fig. 4A–D; Table 4*). These findings support the view that N enrichment reduces host carbon allocation to fungi, weakening mutualistic dependence (Lekberg et al., 2021). Simultaneously, increased N accumulation and decreased phosphorus concentrations in AM absorptive

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roots point to a functional decoupling between fungal morphology and root chemistry (*Figs. 3A, B, 4A–D; Table 3*), suggesting that nutrient acquisition becomes increasingly plant-driven as fungal contribution declines. This imbalance is likely due to the collapse of fungal-mediated phosphorus acquisition capacity, as the suppressed hyphal network limits P foraging while root N uptake becomes increasingly self-sufficient (*Figs. 3B, 4C, D*), a pattern previously observed in N-enriched AM systems (Unger et al., 2021).

This asymmetrical response across mycorrhizal types reveals clear divergence in symbiotic sensitivity (Rúa et al., 2024). AM-associated roots exhibited sharper declines in hyphal proliferation and colonization, which may stem from their reliance on carbon-intensive signaling and absence of external protective structures (Da et al., 2023). ECM roots, by contrast, retained more stable morphological traits and nutrient uptake, possibly due to their capacity for enzymatic mineralization and their thicker sheathing systems that buffer external fluctuations (Addesso et al., 2023). This differentiation supports the concept that ECM symbioses operate under a more conservative carbon economy, preserving fungal integrity under moderate N loads, whereas AM systems rapidly downregulate fungal growth when host carbon investment is constrained (Chaudhary et al., 2025). Similar patterns have been observed in experimental forest systems, where AM fungal abundance and functionality declined sharply in response to elevated N, while ECM assemblages remained structurally intact (Moore et al., 2021; Carrara et al., 2021).

Integrating morphological and chemical trait shifts, our findings underscore a fundamental reconfiguration of the soil-plant-fungus feedback loop under N addition. These changes suggest that nutrient acquisition becomes increasingly plant-driven as fungal contribution declines (Liu et al., 2020). Such divergence between root nutrient status and fungal morphology reflects a decoupling of functional traits, indicating that nutrient assimilation and symbiotic development are no longer synchronized (*Figs. 3A, B, 4A–D*). Reduced phosphorus in AM roots—despite increased N—suggests that the diminished hyphal network restricts P foraging capacity, undermining mutualistic balance and altering nutrient stoichiometry (*Figs. 3B, 4C*, *D*). In ECM systems, however, nutrient accumulation was maintained, reflecting the sustained function of external hyphae despite modest morphological contraction (*Fig. 3A; Table 3*). These outcomes reflect a broader mechanistic pathway: N enrichment reduces host carbon flow, suppresses fungal growth (especially in AM), and reorients nutrient uptake toward direct root assimilation, thereby weakening the reciprocal interdependence of mutualisms and introducing stoichiometric imbalances into the belowground nutrient cycle (*Figs. 3* and *4; Tables 3* and *4*). These findings are consistent with Feng et al. (2022), who demonstrated that N enrichment suppresses microbial carbon demand, and Wu et al. (2023), who emphasized the role of mycorrhizal type in mediating plant responses to N-driven stoichiometric shifts. Notably, the significant interactions among N treatment, root class, and symbiosis type (*Table 4*) highlight the layered ecological filtering shaping these responses.

From an applied perspective, the decoupling of mycorrhizal morphology and root nutrient content under N enrichment signals a loss of long-term nutrient buffering capacity, especially in AM-dominated systems (Messier et al., 2024). Reduced fungal-mediated phosphorus acquisition—despite increased N uptake—suggests a weakening of mutualistic function and accelerated nutrient turnover. These risks are particularly acute where phosphorus cycling depends on hyphal networks (Luo et al., 2024). To mitigate such impacts, management should aim to conserve fungal functionality by limiting N inputs, promoting ECM-associated species, and enhancing soil carbon availability through organic amendments or litter retention (Rembelski et al., 2023).

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N-induced root and fungal trait plasticity reveals functional disintegration of belowground mutualisms

Together, our findings reveal a tightly coupled, trait-mediated feedback between N availability, mycorrhizal identity, and fine-root functional differentiation, which collectively restructure root morphology, nutrient allocation, and fungal development under chronic N deposition. This restructuring ultimately leads to the breakdown of coordinated root-fungus functional integration, as N enrichment uncouples the once-synergistic relationship between symbiotic development and nutrient acquisition (*Figs. 2A–E, 3B, 4A–D; Tables 3* and *4*). The observed divergence in trait plasticity—characterized by ECM transport roots thickening and elongating, AM absorptive roots exhibiting increased SRL and surface area, and concurrent suppression of AM fungal morphology—indicates a form-dependent shift in resource foraging strategies (*Figs. 2A–D, 4B–D*). These patterns converge on a broader mechanism: N enrichment reshapes belowground architecture by altering the balance between structural persistence, nutrient uptake efficiency, and fungal investment (Galindo-Castañeda et al., 2022). AM systems, in particular, exhibit rapid hyphal regression and nutrient decoupling, as increased root N coincides with diminished fungal colonization and phosphorus content (*Figs. 3A, B, 4A–C*), suggesting that mutualistic feedbacks collapse when fungal contributions are no longer beneficial to the host. Similar AM-specific sensitivity has been widely reported, with N-induced suppression of hyphal development and phosphorus transfer frequently observed in both field and experimental systems (Rui et al., 2022; Xie et al., 2022). These changes indicate that N acts not merely as a nutrient input, but as a systemic regulator of trait expression and turnover, disrupting belowground cooperation through stoichiometric imbalance and energetic uncoupling (Liu et al., 2024).

These insights carry significant implications for modeling forest ecosystem responses to global change. Current biogeochemical models often overlook symbiosis-specific root trait syndromes, mycorrhizal feedbacks, and construction cost trade-offs embedded within belowground foraging strategies. The trait-based divergence identified in our study—particularly the asymmetry between absorptive and transport roots across AM and ECM types—underscores the need to disaggregate fine-root pools by topological order and mycorrhizal affiliation rather than by diameter or depth alone (*Fig. 2*; *Table 3*), consistent with recent proposals (Hogan et al., 2020; Huo et al., 2022). Furthermore, the demonstrated decoupling between root nutrient content and fungal morphology (*Figs. 3* and *4*) highlights the importance of explicitly modeling carbon-phosphorus trade-offs and fungal contribution thresholds in nutrient uptake simulations (Allen et al., 2020).

Future studies should move beyond short-term responses to examine the temporal dynamics of root-fungus coordination across seasons or successional stages (Gundale et al., 2021; Santos-Medellín et al., 2023). Combining isotopic labeling, functional genomics, and high-resolution root imaging will help identify thresholds beyond which mutualistic stability collapses (Snyder et al., 2022; Burz et al., 2023). Integrating key traits such as root tissue density, specific root length, hyphal characteristics, and colonization rates into predictive models will be essential for capturing nonlinear responses to N enrichment (Blagodatskaya et al., 2021). Finally, integrating microbial enzymatic profiles and symbiosis-responsive transcriptional markers may further elucidate the biochemical underpinnings of root-fungus disintegration, offering mechanistic insights into carbon-nutrient decoupling under anthropogenic change.

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Conclusion

This study demonstrates that N enrichment modulates belowground resource strategies through coordinated shifts in root traits and mycorrhizal morphology. Through a factorial field experiment integrating N gradients, mycorrhizal types, and root functional classes, we identified four key findings: (i) Fine-root morphology responded in a symbiosis- and function-dependent manner, with ECM transport roots thickening and elongating under high N, while AM absorptive roots exhibited increased SRL and surface area at moderate levels. (ii) Root tissue density declined consistently across all treatments, suggesting a trait-based reduction in construction investment under nutrient enrichment. (iii) N addition significantly suppressed mycorrhizal colonization and hyphal development, particularly in AM associations, leading to diminished fungal-root nutrient integration. (iv) AM roots accumulated more N but less phosphorus under elevated N, indicating a functional decoupling between root nutrient content and fungal foraging capacity.

These findings reveal that chronic N deposition alters belowground mutualisms in a mycorrhizal-type-dependent manner, destabilizing nutrient acquisition strategies and rhizosphere nutrient balance. For forest management, incorporating trait plasticity and mycorrhizal identity into conservation planning is essential for predicting ecosystem resilience and guiding reforestation efforts under atmospheric N loading. Moreover, the demonstrated sensitivity of absorptive and transport roots to N enrichment provides mechanistic insight relevant to slope-stabilization engineering, where root-fungus coordination underpins soil reinforcement. Integrating these trait-based responses into ecological restoration frameworks will therefore be crucial for anticipating nitrogen-driven shifts in forest function and for designing nature-based solutions that enhance soil stability in nutrient-stressed landscapes.

Future research should quantify the thresholds and reversibility of root-fungus decoupling using isotopic tracers and high-resolution trait mapping. Integrating enzymatic activity, carbon allocation dynamics, and trait-based modeling will be critical for forecasting long-term nutrient flux and refining ecosystem-scale predictions under global change.

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