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Root-mycorrhizal foraging strategies shift with forest age more than with nitrogen manipulation

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Abstract

Plant nutrient foraging depends on roots and mycorrhizal fungi, which are affected by plant carbon (C) investment and soil nutrient availability. The C supply for root metabolism and associated fungi might be diminished as the host plant size or age increases, while the quality and quantity of soil nitrogen (N) change with forest age. There is still no holistic understanding of how the organization of belowground mycorrhizal root structure and fungi in the nutrient acquisition continuum shifts with forest age and soil resources, which restrains our understanding of the functional relations among roots, fungi, and soil. Here, we examined shifts in the absorptive root, mycorrhizal strategies, and soil-associated fungal community compositions after 9 years of nitrogen manipulation (0, 20, and 50 kg N ha^{-1} $year^{-1}$) in temperate larch forests across three age cohorts (11, 20, and 45 years). We found that the effect of forest age on root and fungal traits outweighs that of nitrogen treatment. Specifically, with increasing forest tree age, root respiration and specific root length decreased, while protective investments such as tissue density and phenolics increased. Meanwhile, the proportion of ectomycorrhizal fungi of the long-distance exploration type decreased, but those of the short-distance exploration type increased. Together, these patterns suggest a forest age-mediated nutrient acquisition continuum spanning from "explorative roots with long-distance exploration types" to "conservative roots with short-distance exploration types." We propose that

this nutrient acquisition continuum is functionally constrained by the "size vs. rate" trade-off between the root architecture and root segment metabolism, and the "roots vs. mycorrhizal fungi" complementarity between root architecture and mycorrhizal exploration types. Our results suggest that forest age explains shifts in systemic functional trade-offs in root architecture, root segment metabolism, and mycorrhizal exploration types.

KEYWORDS
forest age, metabolism, microbe, mycorrhizal exploration types, nutrient foraging strategies, root architecture

INTRODUCTION

Nutrient foraging strategies are shaped by the traits of roots and mycorrhizal fungi (Chen et al., 2016; Eissenstat et al., 2015; Liu et al., 2015), which are related to the carbon (C) investments in roots or mycorrhizal fungi (Bardgett et al., 2014; Bergmann et al., 2020). Biological growth and C allocation have been predicted to scale with body size or age across a wide range of species (Brown et al., 2004; West et al., 1997, 1999), but to date, the effect of the forest age on the continuum from the root to mycorrhizal hyphae to soil microbes remains less investigated (Hagenbo et al., 2018; Kyaschenko et al., 2017; Martin-Pinto et al., 2022). This knowledge gap makes it difficult to predict the interactions between plant roots and soil fungi, and their collective impact on soil organic C and nitrogen (N) cycling during forest development (Luyssaert et al., 2008; Terrer et al., 2021).

Forest ecosystem function is strongly shaped by tree aging and belowground succession (Anthony et al., 2022; Clemmensen et al., 2015). Nutrient uptake by trees is related to the organization of the nutrient foraging continuum, which mainly consists of three components: root architecture, absorptive root segments, and mycorrhizal fungi. The absorptive root foraging process is inherently linked to plant physiological status (Reich et al., 1998). Plant size or age influences the allocation of energy for growth (Niklas & Enquist, 2001), foraging resources (Albornoz & Lieth, 2015), and defense (Wahl & Ryser, 2000). A small plant has a high surface area-to-volume ratio (Niklas, 1994). It loses heat to its surroundings very quickly and must grow and fix more C to offset this energy loss (Brown et al., 2004) so that more C is allocated to the leaves and absorptive fine roots for acquiring resources (Raich et al., 2014). Furthermore, the nonstructural C quality in a young plant is higher, and most of the nonstructural carbohydrate is respired by new roots (Carbone et al., 2013). In general, the roots of fast-growing young trees demonstrate a series of explorative characteristics, such as rapid proliferation, efficient construction of new tissues (with higher specific root length [SRL], root length of per unit mass, meters per gram) (Eissenstat, 1991; Ryser & Lambers, 1995), and greater respiration (Ceccon et al., 2016). As trees grow, the root architectural traits (i.e., root length density [RLD], total root length per unit soil area, centimeters per square centimeter), which represent size-independent aspects of the root system, are often proportional to increases in the aboveground parts (Makkonen & Helmisaari, 2001; Yuan & Chen, 2012). In large plants, the root system is likely to be large enough to occupy most of the soil space (Tamooh et al., 2008), so more C must be invested for root population maintenance, defense and physical support (Borja et al., 2008), while the metabolism of roots (i.e., SRL and root respiration) tends to be conservative (Rosenvald et al., 2013).

The growth and nutrient foraging by mycorrhizae (including root-associated fungi) largely depend on root metabolism and host plant C supplies (Chen, Koide, & Eissenstat, 2018). More than 20% of C is consumed by ectomycorrhizal (EcM) fungal symbionts (Hobbie, 2006; Hobbie & Hobbie, 2006). Mycorrhizal foraging may be influenced by hyphal exploration types (Anthony et al., 2022). The construction cost, nutrient benefits, and decomposition rates vary with fungal hyphal exploration distance (Agerer, 2001; Fernandez & Kennedy, 2016). Long- or medium-exploration types are generally characterized by rhizomorphs, high enzymatic activity, and extensive soil exploration. In contrast, short-distance and contact exploration types typically acquire N and phosphorus (P) near the root (Agerer, 2001; Tedersoo et al., 2012; Tedersoo & Smith, 2013). However, these generalizations have not been fully tested for consistency across species. Some studies have found that long-distance exploration types were more prevalent in areas of low root density (Peay et al., 2011), such as in

early-aged *Pinus sylvestris* stands (Rudawska et al., 2018) and *Cistus ladanifer*-dominated scrubland (Martin-Pinto et al., 2022). These studies found a pattern of "young forests with long-distance mycorrhizal fungi," which implies that the root system architecture and mycorrhizal fungi hyphal exploration types are complementary in nutrient foraging. Alternatively, other studies found that the abundance of long-distance mycorrhizae was lowest in the youngest plots and increased in the middle-aged (35 years) *Pinus banksiana* forests (Wasyliw & Karst, 2020) or mature *P. sylvestris* forests (Guo et al., 2020) based on a large-scale investigation. Although a complementarity was found to exist between arbuscular mycorrhizal fungi and roots (Eissenstat et al., 2015; Liu et al., 2015), the existing studies have failed to resolve the organization between EcM fungal hyphal exploration type and root system architecture. Understanding the interaction between roots and mycorrhizal fungi is crucial for predicting C flux, nutrient acquisition, and microbial interactions, since EcM fungi colonize ~60% of global tree stems (Brundrett & Tedersoo, 2018; Chen et al., 2016; Steidinger et al., 2019).

The nutrient foraging continuum may also be influenced by the interactions between root- and soil-associated fungi. Both saprotrophic and symbiotic fungi are responsible for decomposition, and they depend on the same nutrient sources and share similar soil space (Lindahl & Tunlid, 2014). The competition between these two fungal types is expected. If soil N plays a key role in this competition, the assembly process of rhizosphere soil saprotrophic fungi may be less affected by root architecture and EcM fungi (Maaroufi et al., 2019), as saprotrophic fungi rely on decomposing organic matter for energy, while EcM fungi mainly depend on plant C supplies. Alternatively, if the forest age-related C metabolic effect rate (characterized by higher respiration rate and fast growth rates in younger or smaller sizes of plants) in root segments plays a greater role in belowground responses, then younger, smaller plants would be expected to allocate more C to their roots (Gong et al., 2012); in return, this would lead to higher mycorrhizal colonization. In this case, EcM fungi gain a competitive advantage over soil saprotrophic fungi (Hagenbo et al., 2018; Kyaschenko et al., 2017). Consequently, the abundance of root-associated fungi may be increased, whereas soil saprotrophic fungi may be reduced. Furthermore, soil N also significantly impacts the composition of EcM fungal communities (Hasselquist & Högberg, 2014). The interactions between root-associated and soil-associated fungi are complex when considering both soil N and plant C. Network analysis allows us to visualize and quantify the interactions among fungal communities, particularly regarding their competition or cooperation. However, how the root–fungi–soil feedback changes with changes in soil N and plant C as the forest develops remains unclear. While the impact of nitrogen on plant roots and soil microbes is acknowledged, few studies have examined how both nitrogen and forest age interact to affect the relationships between roots, mycorrhizal hyphae, and soil microbes. This gap makes it difficult to predict forest nutrient foraging strategies with aging and nitrogen deposition in climate change.

The foraging strategy is likely to be poorly predicted by focusing solely on either the roots or hyphae (Hodge & Storer, 2015); and the generalizations about nutrient foraging patterns from sapling or pot experiments are difficult to interpret, especially given the dynamic nature of symbiosis as roots grow (McNickle et al., 2015). To better understand how the forest age and soil N drive the nutrient foraging continuum, we developed a holistic framework for examining the root architecture, root segments (first-order roots), mycorrhizal strategies, and root and soil fungal community composition. Here, we selected *Larix principis=rupprechtii*, a species extensively planted in northern China; it belongs to the larch genus, which covers nearly 12% of the global forest area (Gauthier et al., 2015; Shakhmatov et al., 2022). Our experiment focused on 11-, 20-, and 45-year-old larch pure forests, representing different developmental stages, with three levels of soil N manipulations. We investigated variations in root architectural and metabolic traits, and mycorrhizal exploration types (Appendix S1: Table S1).

Using this system, we tested three hypotheses: (1) Trade-offs exist between root architectural traits and the physiological metabolic traits of root segments as forest aging. Specifically, lower total absorptive root biomass (aRB) or RLD in young forests is associated with higher root segment respiration. (2) Root architectural traits and mycorrhizal exploration types are complementary in nutrient foraging across different age classes. Younger and smaller trees, with lower root biomass, will explore a larger soil volume by associating with long-distance EcM exploration types, whereas trees with greater root biomass in mature forests rely more on short-distance EcM exploration types. (3) Both forest age and soil N addition significantly influence the interactions between root-associated fungi and soil-associated fungi. A reduction in C inputs to root tips in mature forests reduces the investment in EcM fungi. As a result, mycorrhizal exploration shifts from long-distance to short-distance types, potentially creating a niche for soil saprotrophic fungi, which are further promoted by the accumulating litter (Figure 1).

One aim of this study was to integrate root traits with EcM fungi to improve our understanding of tree ecology

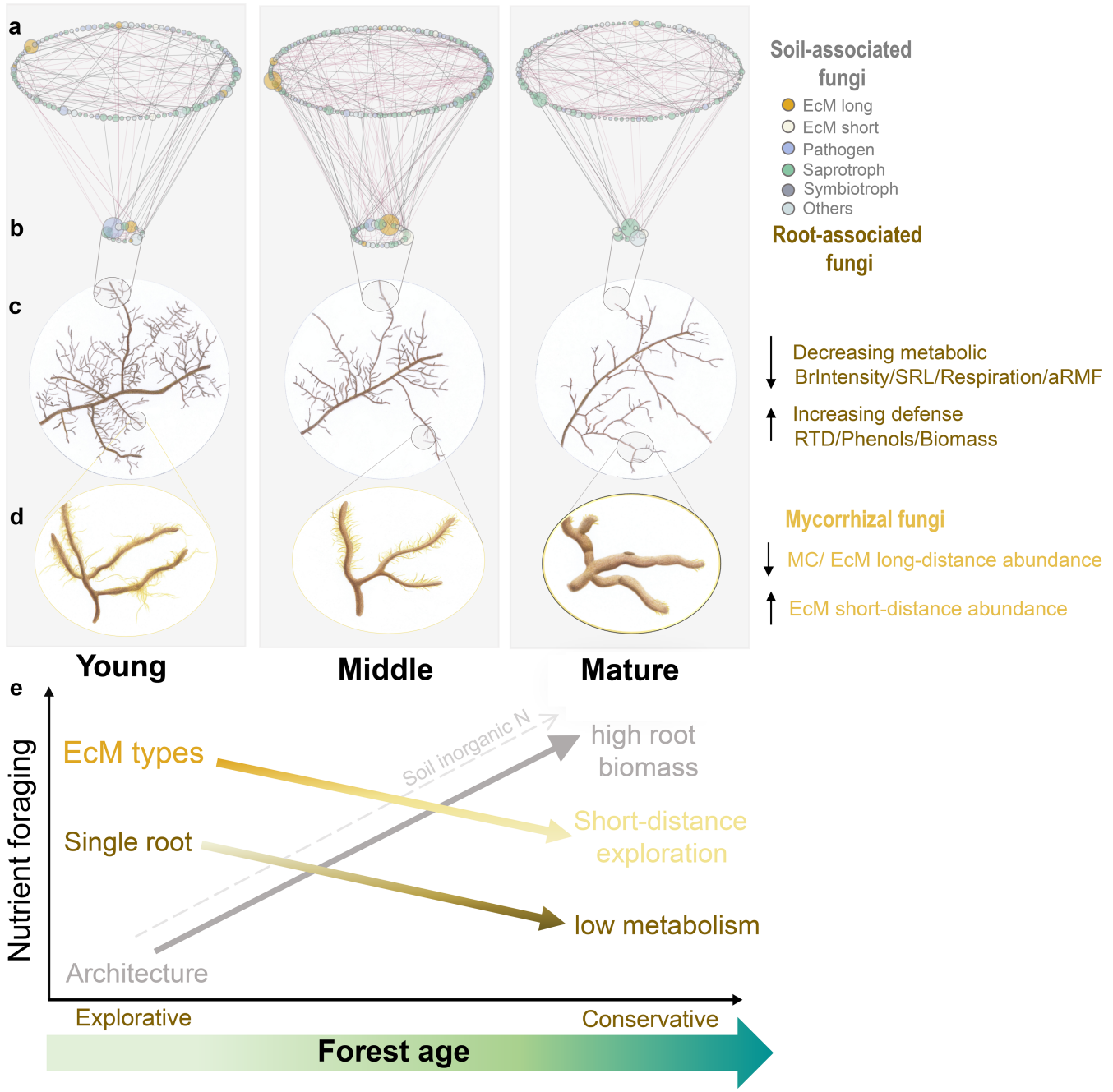

FIGURE 1 Nutrient foraging continuum shifts with stand age in the temperate larch forest. Tree nutrient foraging depends on (c) root and (d) mycorrhizal traits, and (b) their associated fungi and (a) interacts with soil-associated fungi. (e) When the young sapling grows larger, the root system architecture parameters, such as root biomass or root length density, allometrically grow with the shoot (gray line); however, the proportion of carbon investment to resource acquiring organs is likely to decrease as the forest ages, and the root segment metabolic activity, such as respiration, would decline (brown line). As a result, the ectomycorrhizal fungi (EcM) foraging type adapts to the combinations of root carbon supplies and the quantity and quality of soil nutrients, and so the long-distance exploration types are likely to become short-distance types when soil inorganic N accumulates as the forest matures. Overall, the nutrient foraging tends to shift from an "explorative strategy" of young stands to a "conservative strategy" of mature stands. aRMF, absorptive root mass fraction; BrIntensity, branching intensity; MC, EcM colonization; RTD, root tissue density; SRL, specific root length. Illustration credits: Zeqing Ma and Gaigai Ding.

and their associated soil microbes. This innovative approach links root architectural and physiological traits of roots with their mycorrhizal partners, revealing how these interactions co-vary across different age classes. By studying these relationships, we sought to clarify the mechanism of nutrient acquisition strategies in different stages of forests, thereby enhancing our knowledge of belowground forest dynamics and succession.

MATERIALS AND METHODS

Study sites and control experimental design

This study was conducted in larch (*Larix principis-rupprechtii*) plantations of Saihanba Ecological Station of Peking University (42°24′43″ N, 117°14′50″ E, 1505 m above sea level [asl]), Hebei Province, China (Appendix S1: Figure S1). The mean annual precipitation and mean annual temperature are about 450 mm and −1.4°C, respectively. The soil is well drained and classified as sandy soil with ambient N deposition of 13 kg ha^{-1} $year^{-1}$ (Sun et al., 2016).

We established a total of 27 plots for the forest age and N-fertilizer treatments. In August 2009, we selected three larch plantations aged 11, 20, and 45 years to observe the variations in root traits and fungal communities. According to the "Classification of age class and age group of main tree species" issued by the China National Forestry and Grass Administration in 2010, the 11-year-old plantation was classified as a young forest, the 20-year-old plantation as a middle-aged forest, and the 45-year-old plantation as a mature forest. Nine plots were set up in each forest, with each plot covering an area of 20 × 20 m and surrounded by a buffer zone of at least 10 m. The experiment included three levels of N addition (N0: no N addition; N20: 20 kg N ha^{-1} $year^{-1}$; and N50: 50 kg N ha^{-1} $year^{-1}$). Each treatment had three replicate plots that were randomly distributed within each forest stand. N fertilization was applied as a liquid (urea) six times per year during the growing season (May to October) from 2010 to 2018. All three of these stands were in their first rotation after the primary forest was harvested in the last century. The trees in the young, middle-aged, and mature forests were planted in 2000, 1991, and 1966, respectively. No forest management measures were applied in the three stands during the experiment. All three planted stands followed similar climate and soil trajectories. To minimize climatic and soil type differences, the distance among any two stands was less than 2 km. The soil bulk density was 1.47, 1.50, and 1.47 g cm^{-3} in the young, middle-aged, and mature forests, respectively. The total soil nitrogen content was 0.29%, 0.22%, and 0.22% for the respective three age classes (Appendix S1: Table S2). Thus, the differences in root traits, fungal communities, and soil nutrients between stands were mainly attributed to forest stand age or N addition. More details about these controlled experiments are in Yan et al. (2018).

Sampling and storage

We collected the root and bulk soil samples from more than 10 trees in each plot from July to August 2018. The topsoil (0–10 cm) was gently excavated around the base of each tree to expose the lateral roots, and the intact root samples (including more than five root orders) were then extracted following Guo et al. (2008). A total of about 50 root samples with intact branches were collected from each plot. After root collection, a soil auger was used to obtain bulk soil samples at depths of 10 cm, and that sample was taken at a distance of 30 cm from the trunk. The soil collected from each plot was thoroughly homogenized, and a 0.5 kg subsample was isolated to represent the bulk soil for that plot. We obtained a total of 27 bulk soil samples from 27 plots. Root and bulk soil samples were immediately sealed in zip-lock plastic bags and stored in a cooler.

In the laboratory, each set of root branches was shaken to remove loosely adhering soil until no further soil could be detached. Rhizosphere soil, which was firmly attached to the roots, was collected using a sterilized soft-bristled brush (Mendes et al., 2018). The rhizosphere soil from each plot was mixed into a single sample, and a total of 27 rhizosphere soil samples were obtained. Each rhizosphere soil sample was divided into two subsamples. One part was stored at −80°C for high-throughput sequencing of soil-associated fungi, and the other was stored at −20°C for measuring the soil inorganic N concentration. Bulk soil samples were passed through a 2 mm mesh sieve, air-dried, and then analyzed for soil total N and C concentrations and soil available phosphorus (P). Absorptive fine root samples from three to five trees per plot were washed with water and immediately stored in formalin-aceto-alcohol (FAA) solution (containing 90 mL 50% ethanol v/v, 5 mL 100% glacial acetic acid v/v, and 5 mL 37% formaldehyde v/v) for measuring EcM colonization. Five washed intact roots from each plot were randomly selected and stored at −20°C for measuring root architecture (i.e., branching intensity). The remaining root samples were dissected with fine forceps based on the methods of Pregitzer et al. (2002) and Guo et al. (2008). All first-order roots were thoroughly homogenized for each plot. From this homogenized mixture, at least 300 first-order roots were randomly selected per plot and stored at −80°C for high-throughput sequencing of root-associated fungi. In parallel, sufficient first-order root samples were stored at −20°C for analyzing their morphological and chemical traits.

Trait measurements

The first-order roots were scanned as images at a resolution of 400 dpi with a scanner (Seiko Epson Corp., 10000 XL Suwa, Japan). Based on these images, we obtained the average root diameter (Diam), total root length, and

root volume by using WinRHIZO software (Regent Instruments Inc., Québec City, Canada) (Chen et al., 2016). Then these root samples were oven-dried at 65°C for 48 h to obtain the dry mass. SRL was calculated as the ratio of total root length to root dry mass. Root tissue density (RTD) was determined by the ratio of root dry mass to root volume. RLD was calculated by multiplying the SRL by aRB within a soil core. Root branching intensity (BrIntensity) was calculated as the number of first-order root tips divided by the total root length of the corresponding second-order roots. After the roots were crushed by a bead mill tissue grinder, we determined the total root phenolic content (Phenols) according to the absorbance of the chromogenic reaction with Folin-Ciocalteu reagent at 765 nm by spectrophotometry (SpectraMax 190 Elisa, USA) (Ainsworth & Gillespie, 2007). We defined the EcM colonization (MC) as the proportion of colonized root tips relative to the total number of observed root tips (Kong et al., 2014). Specifically, at least 100 first-order root tips were randomly selected from the root samples stored in the FAA solution. The samples were rinsed with deionized water and the numbers of root tips colonized (i.e., root covered by fungal sheath) and non-colonized by the fungi were recorded during observations with a 30× stereoscope (Leica S9I El, Germany) (Kong et al., 2014).

Root function measurement: Respiration

We measured the absorptive root respiration rate by in vitro root methods (Nakamura & Nakamura, 2016) with a portable dissolved oxygen meter (WTW, Multi 3510IDS Germany). In August 2018, three bottles (200 mL) of amber glass with a wide mouth packed with distilled water and total nutrient admixture in a 1:1 ratio (v/v) were placed in each plot. All three bottles were oxygenated by an oxygen pump until saturation, to ensure no change in the oxygen concentration. After preparing the bottles, we collected live absorptive fine roots from five trees in each plot, gently washed them with deionized water, and placed them into the bottles, which were then capped. After a 20 min incubation, we measured oxygen concentration using a dissolved oxygen meter. We conducted these experiments from 8 a.m. to 10 a.m. to ensure relatively similar environments and to avoid exposure to sunlight. The entire process, including root sampling, cleaning, and separating, was finished within 3 min to avoid the rapid decline in respiration after root isolation. Then root biomass (B_{root}) was determined by oven-drying in the laboratory.

$$R_{root} = (O_1 - O_2)/(B_{root} \times T)/M,$$

where R_{root} (in nanomoles per gram per second) is the absorptive root respiration; O_1 (in milligrams per liter) is the saturated oxygen concentration of the solution at the starting point; O_2 (milligrams per liter) is the oxygen concentration of the solution after the root has respired; B_{root} (in grams) is the dry mass of absorptive roots in the bottle; T (in minutes) is the duration time of respiration by the root, which was 20 min in this study; and M is the molar mass of oxygen (32 g mol^{-1}).

Biomass allocation

To measure the absorptive root biomass (aRB) within the top 10 cm of soil, we collected 12 soil cores (3 cm diameter, 10 cm depth) randomly from each plot using a soil auger in July 2018. The roots were manually removed from a total of 324 soil cores across the 27 plots. Then, we separated live absorptive fine roots (including the first two orders) from the root samples. The absorptive fine roots were cleaned and oven dried at 65°C for 48 h to obtain a constant mass. We investigated the distribution of absorptive roots in the 0–50 cm soil layer, and almost 50% of them were found to be distributed in the 0–10 cm layer (Appendix S1: Figure S2). To better match the soil properties and soil microorganisms, our analysis focused on aRB in the top 10 cm of soil.

Leaf biomass was estimated using two litter traps (1 m × 1 m) in each plot from 2014 to 2016 (Yan et al., 2018). We measured stand density, the dbh, and tree height for 15–20 individual trees in each plot and used allometric equations of larch in this site (Sun et al., 2016) to estimate the biomass of branches and stems. Biomass allometric models often do not account for the tiny absorptive roots that are mainly concentrated in the topsoil. Therefore, we estimated total root biomass in two parts: the absorptive root part from direct measurement (as described above) and the coarse root part using an allometric equation. Root mass fraction (RMF) was calculated as the percentage of total root biomass to whole plant biomass. The absorptive root mass fraction (aRMF) was the aRB divided by the total plant biomass at the stand level.

Measurements of the root- and soil-associated fungal communities

The fungal community compositions of first-order root and rhizosphere soil samples were analyzed by next-generation amplicon sequencing. In each plot, we collected more than 300 first-order root tips and freeze-dried them at −80°C. These roots were ground

into powder with liquid N added continuously during the grinding process to ensure that the roots remained in a frozen state. From the powered root samples, we extracted 300 mg of tissue for analysis. We prepared a total of 27 samples of root tissues corresponding to 27 samples of rhizosphere soil. We extracted the total DNA from the root and soil samples by using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Fungal genes were amplified with the forward primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and the reverse primer ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) using an ABI GeneAmp 9700 polymerase chain reaction (PCR) thermocycler (ABI, CA, USA). Sample-specific 6 bp barcodes were incorporated into the primers for multiplex sequencing. The PCR amplification was performed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, and a single extension at 72°C for 10 min, ending at 10°C. The final volume of the PCR mix was 20 μL, including 2 μL 10 × PCR Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL Forward Primer (5 μM), 0.8 μL Reverse Primer (5 μM), 10 ng of Template DNA, 0.2 μL rTaq DNA Polymerase (5 U/μl), 0.2 μL BSA, and ddH_2O added up to 20 μL. The PCR reactions were conducted in triplicate. The PCR products were extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a Quantus Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on a MiSeq PE300 platform according to standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

The raw sequencing reads (439,844,377) were demultiplexed, quality-filtered using fastp version 0.20.0 (Chen, Zhou, et al., 2018), and merged using FLASH version 1.2.7 (Magoc & Salzberg, 2011) with the following criteria: the 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded; reads containing ambiguous characters were also discarded, and only overlapping sequences longer than 10 bp were assembled according to their overlapping sequence. The maximum mismatch ratio of the overlap region was 0.2. Reads that could not be assembled were discarded; sequences in each sample were separated according to barcodes (exactly matching) and primers (allowing 2 nucleotide mismatches), and reads containing ambiguous bases were removed. These operations resulted in a final set of 1.46 million sequences (a total of 421,522,494 reads) across 27 root samples. To standardize the sequencing depth across samples, fungal read numbers per sample were rarefied to the smallest number of reads (Bellis et al., 2022). The subsequent statistical analysis used a randomly selected subset of 34,384 valid sequences for each sample (for a total of 928,368 sequences across the 27 root samples). Rarefaction curves are used to determine how much a given sample captures the overall fungal diversity (Lajoie & Kembel, 2021). Accumulation curves showed a plateau for each rarefied dataset, demonstrating that most microbial diversity was included in our analyses (Appendix S1: Figure S3a). The processing of soil samples was similar to the root samples. The subsequent statistical soil analysis used a randomly selected subset of 54,146 valid sequences for each soil sample. Accumulation curves are shown in Appendix S1: Figure S3b. The resulting sequences were clustered into operational taxonomic units (OTUs) using UPARSE at 97% identity, and chimeric sequences were identified and removed using UCHIME (Edgar, 2013). Finally, we obtained 402 and 3799 OTUs from the 27 root and soil samples, respectively. Representative sequences of each OTU were searched against the UNITE database (Koljalg et al., 2005) with the following criteria for a match: sequence similarity ≥ 97%; query coverage ≥ 95%; and *e*-value $< 1 \times e^{-50}$. When the results returned poor matches (< 97% similarities), we defined the OTUs as unclassified (others). We annotated the species classification of each sequence using the RDP classifier. The relative abundance tables for taxa (OTU, genus, or other levels) were generated based on the read count for each taxon across samples using the total-sum scaling (TSS) method.

The functional fungal guilds are assigned with FUNGuild (Nguyen et al., 2016). Of the identified fungal OTUs, 65% of the root-associated fungal OTUs and 50% of the soil-associated fungal OTUs could be assigned to different guilds, including EcM mutualists, saprotrophs, pathotrophs, and symbiotrophs. We further searched fungal functions (at a general level) and lifestyles in FungalTraits (Põlme et al., 2021) to assign some OTUs that were assigned to more than one functional guild. We manually matched OTUs that were unclassified in the functional groups of root-associated and soil-associated fungi using the massBLASTer in the PlutoF system. After that, OTUs that could not be confidently assigned to a specific guild were excluded from the analysis of functional fungal guilds. The relative abundance of the fungal guilds was calculated at the genus level (Appendix S1: Figure S4). EcM exploration types were classified according to Lilleskov et al. (2011) and Tedersoo and Smith (2013). In this study, the exploration types were

classified according to whether the fungal genus forms rhizomorphs. During the pre-experiment period, we observed a total of 540 root tips with a stereo microscope across the 27 plots, and 87% of the mycorrhizal morphology in young forests had long-distance foraging rhizomorphs. In contrast, in mature forests, fewer root tips were colonized by mycorrhizae, and only 1% of the mycorrhizal morphology formed rhizomorphs (Appendix S1: Figure S5a). We noted a remarkable difference between the smooth and fringe/mat types within the medium-distance exploration types, and the latter form far more biomass and chords than the former (Jörgensen et al., 2022). The medium-smooth exploration type resembles the short-distance type, demonstrating lower hyphal biomass. In the mature forests, we observed root tips with rhizomorphs in three out of nine plots, with only 1% of the root tips in each plot displaying the formation of rhizomorphs (Appendix S1: Figure S6). These rhizomorphs appeared undifferentiated and were characterized by smooth mantles or only a few emanating hyphae (Appendix S1: Figure S6). Therefore, we classified fungal genera that fall in contact, short-distance, and medium-smooth into the "short-distance exploration type" due to their lack of rhizomorph formation or lower biomass. Fungi belonging to the medium-distance fringe/mat and long-distance categories were classified as the "long-distance exploration type" because they can form rhizomorphs with high biomass.

Measurements of soil nutrients

Inorganic N (including NH_4^+-N and NO_3^--N) was extracted from 5 g samples of rhizosphere soil with KCl (50 mL of 1 mol L^{-1}), and concentrations were determined using an AA3 continuous flow analyzer (Seal, Norderstedt, Germany) (Kodama et al., 2014). We ground air-dried bulk soil samples (2000 rpm, four cycles, 90 s/cycle), weighed 50 mg of the soil, and determined the soil total N and C contents by using an elemental analyzer (Vario EL III, Elementar, Hanau, Germany) (McGeehan & Naylor, 2008). Soil available P was extracted from 3.0 g of bulk soil with NH_4F-HCl and measured colorimetrically using a visible spectrophotometer (Shimadzu, UV-1900i, Japan) (Tiyapongpattana et al., 2004).

Statistical analyses

We used a linear mixed-effects model (LMM) to assess the effects of N addition, stand age, and their interactions on absorptive root traits, fungal communities, and soil nutrients. Each model included stand age (Age), N addition (N), and their interactions as fixed effects, with a random slope for N across plots, specified by (N|plot). Data were log-transformed prior to variance analysis when necessary.

To better understand the relationships between the drivers (root traits, soil nutrients, and forest age) and the relative abundance of root- and soil-associated fungal functional groups, we employed the canonical correspondence analysis (CCA) using the "vegan" package. A total of 12 variables were used in the CCA to explain the patterns of variations in the root- and soil-associated fungal communities. The CCA was analyzed by Monte Carlo permutation tests (9999 permutations under the full model) with forward selection of explanatory variables (Clemmensen et al., 2015). Variance partitioning analysis was performed to quantify the variation explained by root traits and soil nutrient factors in root- and soil-associated fungal functional groups. Random forest analysis (ntree = 500, nrep = 1000) was conducted to determine the relative importance of root traits and soil nutrient variables in the relative abundance of specific fungal functional groups using the "rfPermute" package (Jiao et al., 2018).

Soil-associated fungi (rhizosphere soil fungi) exhibit a close phylogenetic relationship with root-associated fungi (including rhizoplane and endosphere fungi), particularly saprotrophic fungi within the soil and EcM fungi in roots. These interactions would further influence the foraging strategy of root-mycorrhizal associations. To test our third hypothesis, we analyzed networks of interactions among root- and soil-associated fungi for each stand age and N treatment using the "ggClusterNet" package (Wen et al., 2022). The Hellinger transformation was applied to the relative abundances of OTUs for both root-associated and soil-associated fungi. The interactions among root-associated fungi, soil-associated fungi, and between these two groups were determined using a network co-occurrence analysis. This analysis focused on OTUs with a relative abundance > 0.05% and those that occurred in more than 80% of the total samples (Faust, 2021). Each dot represents a genus of fungi, and the links represent statistical significance ($p < 0.05$). Spearman's correlations had a correlation coefficient > 0.8. Network analysis was visualized with the "ggplot2" package. Most data analyses and diagrams were generated in R (4.1.3).

RESULTS

Optimum balance between root architecture and root segment metabolism

We revealed trade-offs between the root architectural traits (aRB, RLD, and RMF) and root segment metabolism

(root respiration [R_{root}] and SRL) as the larch forest age progressed from the young to mature stages (Figure 2a–f; Appendix S1: Tables S3 and S4). Root respiration decreased from 28.9 to 20.3 nmol g^{-1} s^{-1} ($p < 0.001$) and SRL decreased from 47.0 to 34.7 m g^{-1} ($p < 0.001$) from the young to mature stages (Appendix S1: Figure S7f,g and Table S4), while aRB and RLD increased from 58.4 to 247.9 g m^{-2} ($p < 0.001$), and 27.23–86.0 cm cm^{-2} ($p < 0.001$), respectively (Appendix S1: Figure S7b,d and Table S4). As the forests matured, RTD and phenolic content increased significantly (Appendix S1: Figure S7h,i and Table S4). Meanwhile, the aRB increased four-fold (Appendix S1:

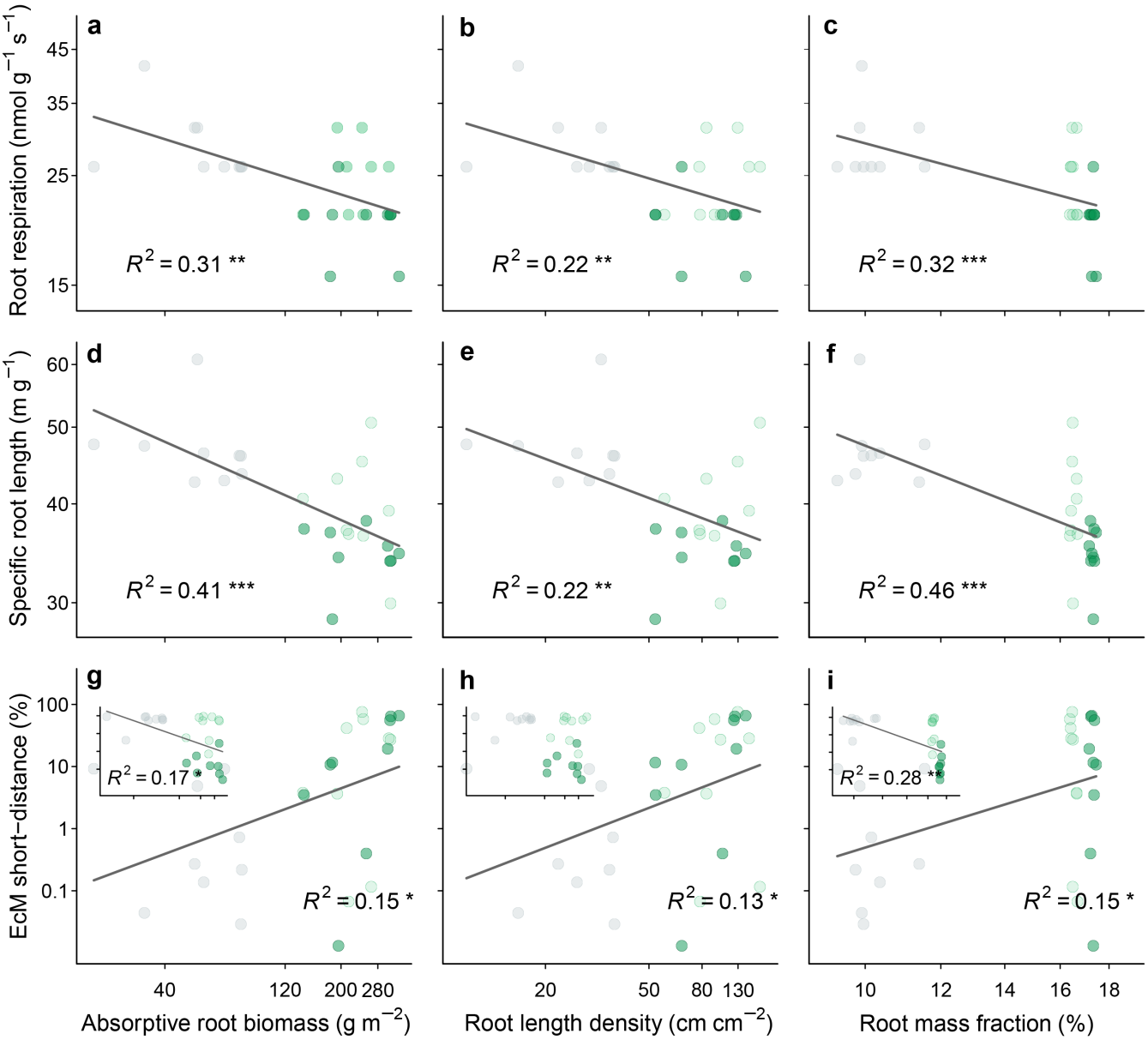

FIGURE 2 Root architecture constrains the metabolic rate of root segments and the exploration type of mycorrhizal fungi during larch forest development. (a–c) Metabolic rate (root segment) versus size (root system architecture) trade-offs in larch forest development, that is, negative relationships between absorptive root respiration versus root architectural traits (absorptive root biomass, absorptive root length density, and root mass fraction). (c–e) Root segment acquisition efficiency (specific root length) versus size trade-offs in larch forest development, that is, negative relationships between the specific root length and root architectural traits in the young, middle-aged, and mature stands. (g–i) Testing for complementarity between exploration types of ectomycorrhizal fungi and root architecture among the three age classes of larch forest. A greater root system size corresponds to a higher abundance of short-distance mycorrhizas, and the young forest with less root biomass compensates by an increase in the long-distance exploration type of ectomycorrhizal fungi (EcM long type) (inset panels). Gray, light green, and dark green represent the young, middle-aged, and mature forest developmental stages, respectively. Data were $\log_{10}$ transformed. Significance of trade-offs: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure S7b), while the absorptive root tips decreased by nearly 72% (Appendix S1: Figure S7a). These results suggested that the root strategy shifted from explorative to more conservative strategies, with increasing root system size during forest development.

The effect of forest age on root traits outweighed that of N addition (Appendix S1: Table S4). Root architectural traits had higher plasticity, as indicated by greater variation (mean CV = 46%) compared to other root traits (Appendix S1: Table S3). The fungal composition and CCA revealed that aRB and RMF were the most important root traits in explaining the shift in fungal functional groups (Figures 3 and 4; Appendix S1: Table S5), suggesting that root architecture may be the leading dimension for predicting foraging continuums as compared to either root morphology or root metabolism.

Shifts in EcM exploration types from young to mature plantations

Mycorrhizal exploration strategy significantly changed with stand age but not with N addition (Appendix S1: Table S4; Figure 3a,b). The relative abundance of short-distance exploration fungi, mainly *Wilcoxina* and *Tomentella*, increased by more than nine-fold from young to middle-aged and mature forests (Figure 3b), despite a ~two-fold reduction in mycorrhizal colonization

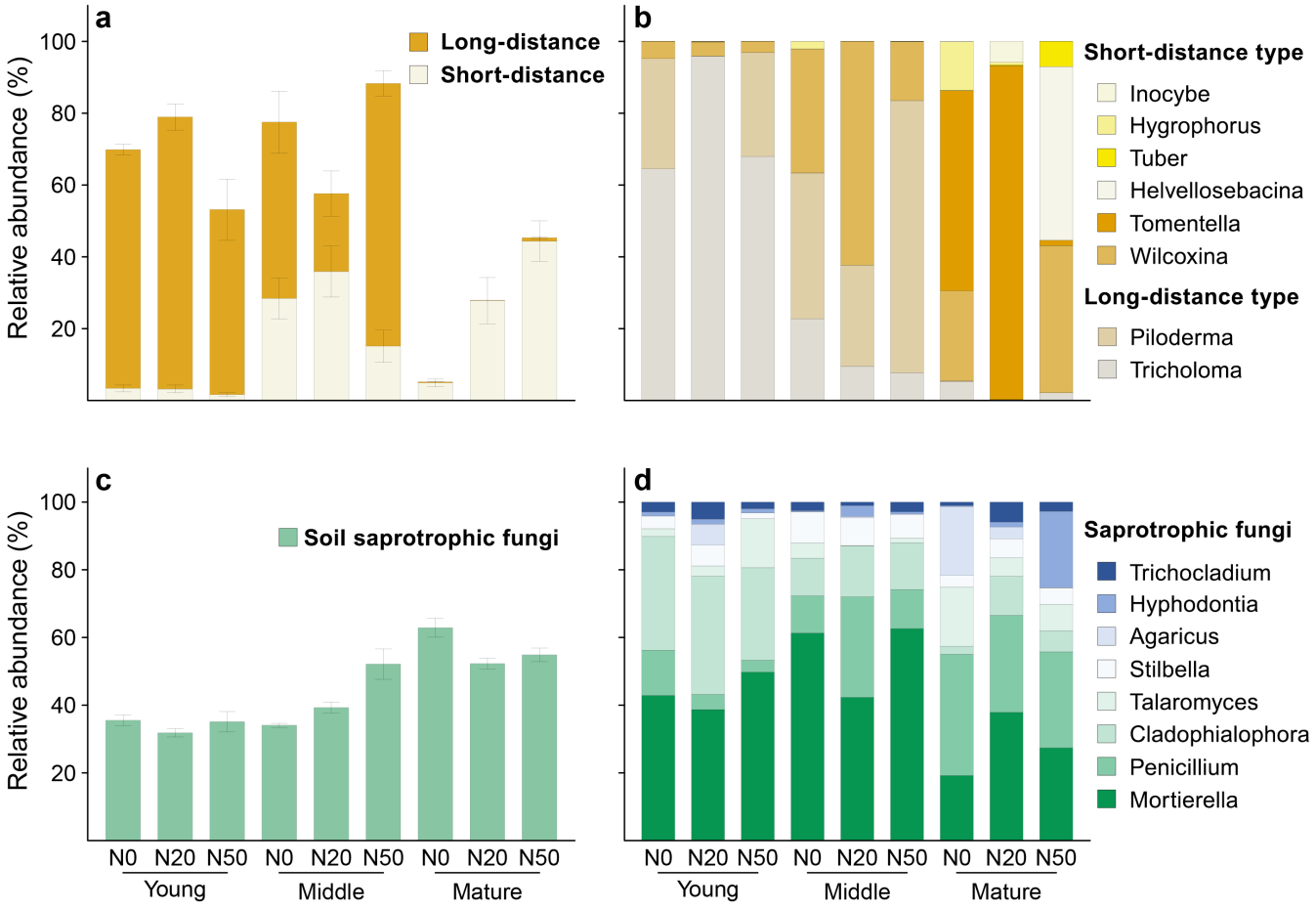

FIGURE 3 The relative abundances of root-associated mycorrhizal fungi and their top eight genera (a, b) and soil-associated saprotrophic fungi and the top eight genera (c, d) are strongly constrained by forest age rather than nitrogen (N) addition across 27 plots in larch forests. Forest age significantly influenced the (a, b) root-associated mycorrhizal fungal communities and (c, d) soil-associated saprotrophic fungal communities. (a) The long-distance exploration type predominantly occupied the young forest, (b) which included genera *Tricholoma* and *Piloderma*, while the short-distance exploration type, which included genera *Wilcoxina* and *Tomentella*, played a dominant role in the mature forest, despite the reduction in the relative abundance of total ectomycorrhizal (EcM) fungi from 71% (average of relative abundance in young and middle-aged forests) to 26% within the roots. The soil-associated fungal community was relatively less affected by forest age or N conditions, as (c) the relative abundance of saprotrophic fungi (green) slightly increased with forest age, while (d) within the saprotrophic fungal functional type, the dominance of *Mortierella* decreased and community evenness increased with aging. Detailed results are presented in Appendix S1: Table S4. Nitrogen treatments: N0, no N addition; N20, 20 kg N ha^{-1} $year^{-1}$; and N50, 50 kg N ha^{-1} $year^{-1}$.

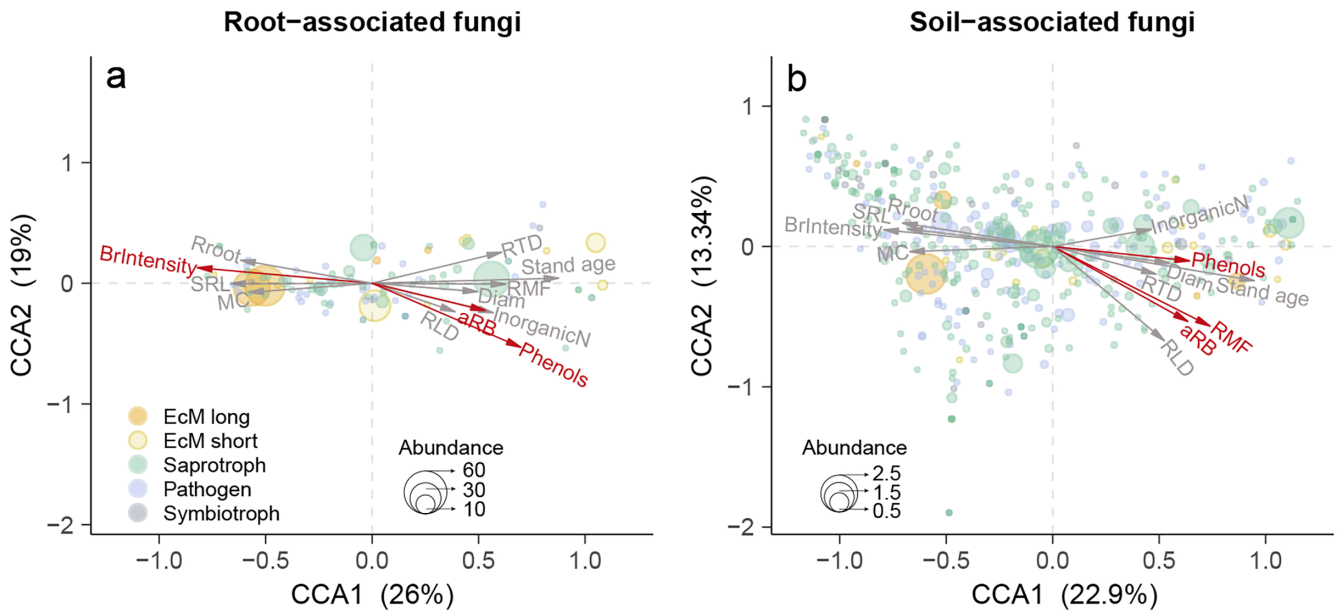

FIGURE 4 Canonical correspondence analysis (CCA) indicating that the root architectural characteristics (branching intensity, absorptive root biomass [aRB], root mass fraction [RMF]) are the leading dimensions in the variation in the fungal community composition of (a) root- and (b) soil-associated fungi in a temperate larch forest. The CCA demonstrated that various combinations of root architectural (branching intensity [BrIntensity], aRB, and RMF) and absorptive root segmental traits (root diameter [Diam], specific root length [SRL], root total phenolic content [Phenols], and root tissue density [RTD]), which correspond to multiple nutrient capture strategies, influenced the fungal community composition within and outside of the roots under different soil N availability levels during forest aging. Detailed trait definitions and abbreviations are provided in Appendix S1: Table S1. The red arrows indicate the selected explanatory variables with $p < 0.10$ by forward selection according to Appendix S1: Table S5. Symbol size is proportional to the average relative abundance of each guild. The circle symbols are colored according to the four fungal functional guilds, and symbol size is proportional to the mean relative abundance. MC, EcM colonization; RLD, root length density; R_{root}, absorptive root respiration.

(Appendix S1: Figure S7j). In contrast, the relative abundance of long-distance exploration fungi, mainly the genera *Piloderma* and *Tricholoma*, decreased from 65% in young forests to 0.5% in mature forests (Figure 3a,b).

Mature forests with a larger root system size had a greater absorptive root occupying more soil volume, and it was complemented by short-distance EcM fungi to forage nutrients (Figure 2g–i). In contrast, young trees with smaller root systems had fewer roots to occupy the soil, mainly through symbiosis with long-distance mycorrhizal fungi (Figure 2g–i, inset panels), which increased the total absorptive length and improved the tree's ability to explore more nutrient patches. Indeed, short-distance EcM fungi and saprotrophic fungi were associated with the roots that had high phenolic content and low root respiration in mature larch forests (Figures 3 and 4; Appendix S1: Figure S8), while the relative abundance of long-distance exploration types of EcM fungi co-varied with greater branching intensity (BrIntensity) and more rapid metabolic activity in the root segments (Figure 4).

The interaction of root- and soil-associated fungi shifted with forest age and were less affected by total soil N

The strength of interactions between root- and soil-associated fungi significantly increased as the forest developed, with the relationship shifting from negative co-occurrence in young stands to positive co-occurrence in middle-aged and mature forests (Appendix S1: Figure S9a and Table S6). The relative abundance of soil saprotrophic fungi increased from 34% in young forests to 57% in mature forests (Figure 3c), while the relative abundance of root-associated EcM fungi declined from 67% in young and 74% in middle-aged forests to 26% in mature forests (Figure 3a). The soil NH_4^+-N was significantly correlated with soil saprotrophic fungi and EcM fungi (Appendix S1: Figure S10c–f). Changes in the relative abundance of functional groups of root-associated fungi were mainly explained by root traits and soil nutrients (aRB, BrIntensity, total phenolic content [Phenols], and soil C:N ratio [SoilCN]), while soil-associated fungi were explained by soil nutrients (Appendix S1: Figure S11).

Network analysis revealed that the number of links between root- and soil-associated fungi in young, middle-aged, and mature forests was 27, 77, and 26, respectively (Appendix S1: Table S6), suggesting that the complexity of networks peaked in the middle-aged forest (Appendix S1: Figure S9a and Table S6). Root- and soil-associated fungi and their interaction changed with stand age, and they were relatively less affected by N conditions (Appendix S1: Figure S9).

DISCUSSION

The shift in nutrient foraging strategy with forest age is a complex adaptive process. We found that the nutrient acquisition continuum was functionally constrained by a trade-off between size-related root architectural traits and root segment metabolism, and by a complementarity between root architecture and mycorrhizal exploration types. These two interactions led to a negative correlation between EcM fungi and soil saprotrophic fungi during forest development, which may be attributed to age-related C allocation and the increase in soil inorganic N content.

The trade-off between size-related root architectural traits and root segment metabolism

Root architecture primarily drives the shift in nutrient foraging with stand age (Figure 4; Appendix S1: Figure S8 and Table S4). Root branching intensity declined considerably, and root biomass increased with stand age (Appendix S1: Figure S7a,b and Table S7). Across different species, root branching architecture and root diameter represent two independent dimensions of nutrient absorptive capacity (Kong et al., 2014). However, within a species, the root diameter of the first order tends to be phylogenetically conservative (Ma et al., 2018). Thus, the root architectural traits are plastic and mainly respond to soil resource supplies (Fitter, 1987; Hodge, 2004), serving as a leading dimension of the nutrient acquisition strategy. In a young forest, to occupy new soil space and acquire P- and N-rich hotspot patches, roots with intensive branching will quickly increase in total root length and the exploited soil volume (de Kroon et al., 2012; Robinson et al., 1999). More than 65% of root tips are colonized by EcM in young stands (Appendix S1: Figure S7j), so a small root system with more hyphae improves nutrient foraging efficiency in the unevenly distributed soil resources. As the forest ages, the root system's horizontal expansion ceases under multiple biotic constraints (de Kroon et al., 2012). With the increasing shift of more nutrients from the soil to litter or plant tissue (Jobbagy & Jackson, 2004), a larger shallow root system with low EcM colonization (MC = 34% in mature forest) can economically exploit the enriched patches to acquire nutrients (Hodge, 2004). Overall, improving nutrient acquisition efficiency likely depends on root architecture plasticity (Freschet et al., 2015; Weemstra et al., 2017), and the plant will deliver root tips into bulk soil patches when the plant size is smaller and communities have less biotic competition.

Tree age modulates root segments with C supplies, promoting trade-offs between the size-related root architectural traits and the metabolic activity of root segments. We focused on the first-order root (root tips), which is the most fragile, N-rich, and metabolically active segment. The root phenolics and tissue density significantly increased with stand age (Appendix S1: Figure S7h,i). The corresponding root respiration and SRL decreased (Appendix S1: Figure S7f,g), consistent with previous studies (Ren et al., 2023; Rosenvald et al., 2013). These changes in root segments follow the metabolic theory (Brown et al., 2004). Young larch forests have higher SRL and respiration, as well as lower RTD and phenolic concentrations, indicating high physiological activity for fast growth (Comas et al., 2002; Rosenvald et al., 2013; Ryser & Lambers, 1995). When the plant size becomes larger, the whole-plant C allocation strategies significantly shift with age (Poorter et al., 2012). First, the size of the root system increases in proportion to the increase in whole-plant size (Appendix S1: Figure S7c), while the root segment metabolic rates decrease (Figure 2a–f). Second, C is allocated to multiple functions, such as storage, anchorage, and reproduction, in addition to growth and absorption (Weiner, 2004). Third, root-root competition within and across species, as well as root defense against herbivores and pathogens, increases with ecosystem development (de Kroon et al., 2012). The relative abundance of pathogenic fungi in both root-associated fungi and soil-associated fungi decreased significantly in mature forests (Appendix S1: Table S4). Under physical and biotic constraints, the expansion of root system size in mature forests may be constrained (McConnaughay & Bazzaz, 1992). A large root system architecture occupying a larger soil space leads to a greater length, harder features (higher RTD), and longer lifespan of the roots (Eissenstat & Yanai, 1997) in mature forests. These shifts in root strategy—from explorative and competitive toward more conservative and tolerant—are reflected in terms of realistically efficient covarying combinations between the architecture (i.e., aRB and RLD) and segments (i.e., SRL and root respiration) of the roots. Our results may reflect the accumulating effect of stand aging,

not only the simple snapshots of young, middle-aged, and mature forests.

We found that adding nitrogen (N) had little effect on the root traits of root respiration and mycorrhizal colonization. Root N may have increased slightly as soil N rose (Zhao et al., 2022), but the first-order roots, which were already saturated with N, did not show significant changes (Burton et al., 2011). Furthermore, the short-term effect of N addition might not be comparable to the long-term accumulated effect of forest age (Appendix S1: Table S4). N addition appears to have a dose-dependent effect (Noguchi et al., 2013), in which N is quickly taken up by the understory initially (Qu et al., 2019), but it eventually leaches or volatilizes, particularly in this sandy soil (Cevallos et al., 2015). As a result, the amount of N the roots ultimately received may have been less than expected. In addition, EcM larch is part of the organic nutrient economy (Phillips et al., 2013), meaning that the root traits may not be sensitive to inorganic N; however, EcM fungi can quickly respond to N (Appendix S1: Table S4, Figure S12). Thus, mycorrhizal colonization (MC) was highest at the medium N level ($p = 0.03$), suggesting that both carbon (C) and nitrogen (N) influence mycorrhizal-root strategies.

Complementarity between root architecture and mycorrhizal exploration types

Nutrient foraging strategies can be defined along a spectrum, from "young explorative roots with long mycorrhizas" to "mature conservative roots with short mycorrhizas." The proportion of EcM short-distance exploration types increased from young to mature larch forests (Figure 3a). This finding is consistent with patterns obtained in *P. sylvestris* (Kyaschenko et al., 2017; Rudawska et al., 2018) and *C. ladanifer*-dominated scrubland (Martin-Pinto et al., 2022). As the root system grows, we observed complementarity between root architecture and mycorrhizal exploration types (Figure 2g–i). Peay et al. (2011) also confirmed that low RLD is complemented by extensive or long-distance exploration types of EcM. Indeed, the low RLD in young forests allows long-distance types to extend (Geml, 2019); while in mature forests, short or contact exploration types are more prevalent (Kyaschenko et al., 2017; Martin-Pinto et al., 2022; Rudawska et al., 2018). At least three reasons may explain why the short EcM fungi gradually become dominant as the root system size expands. First, a large root system combined with short hyphae increases the potential for soil exploitation. A large root biomass or density occupies the soil matrix efficiently, and the short-distance (e.g., contact, short-distance, medium-distance smooth) exploration types maximize a large area of hydrophilic hyphae, which can more rapidly take up inorganic N compared to long-distance types (Lilleskov et al., 2011). Second, potential enzymatic activities in short-distance types are lower than those in long-distance types (Kyaschenko et al., 2017; Tedersoo et al., 2012), which reduces the construction and maintenance cost. Third, hydrophilic hyphae in short-distance types should be favored in stable soil moisture environments when the forest canopy is closed (Geml et al., 2017).

The trend in EcM exploration types observed in this study contradicts the observations in *P. banksiana* (Wasyliw & Karst, 2020) and *P. sylvestris* (Guo et al., 2020; Hagenbo et al., 2018), in which no trends in the abundance of mycelial exploration types were found with increasing forest age. This inconsistency may be due to differences in whether the inorganic or organic N increased with age (Leduc et al., 2013). In our sites, soil inorganic N increased, but total N decreased as the forest aged (Appendix S1: Figure S10a,b), so short-distance EcM types with considerable surface area could easily capture the inorganic N with high mobility (Geml et al., 2017). If organic N is enriched in the mature or older forests, the long-distance exploration types may have an advantage due to their retained decomposition capacities and improved ability to search for hotspots (Anthony et al., 2022). This nutrient acquisition continuum formed by root-mycorrhizal fungi may also be adapted to natural forest succession, which depends on soil N dynamics over time. This inconsistency may also be linked to methodological differences, that is, a potential bias due to the sampling scales and intensities in investigations, which are not like controlled experiments (Lilleskov et al., 2004). EcM fungi may adopt different exploration strategies in different environments even for the same fungal species (Jörgensen et al., 2021). We mainly relied on a molecular approach to define EcM exploration types (Appendix S1: Figure S12a), and our morphological analysis of mycelium (Appendix S1: Figures S5 and S6), and in-growth mesh bags of mycelium production (Appendix S1: Figure S12b) further corroborated our key findings. In young forests, 87% of mycorrhizal morphology had long-distance rhizomorphs, while in mature forests, fewer root tips were colonized by mycorrhiza, and 99% of mycorrhizal morphology had only a few emanating hyphae, or a voluminous envelope of emanating hyphae without rhizomorphs (Appendix S1: Figure S5). Our results suggest that short-distance and contact EcM fungi dominate in mature forests; but these patterns may change as forests continue to age and reach maturity under differing nutrient limitation and pathogen

exposures. For instance, recent research has found that mature forests may rely on root exudation to "mine" nutrients (Wang et al., 2024). Overall, our holistic framework resolved the nutrient acquisition continuum strategies, which shifted from young explorative competitive roots with long mycorrhizae to mature conservative tolerant roots with short mycorrhizae.

Interactions between root-associated and soil-associated fungi

Forest age prompted negative relationships between EcM fungi and soil saprotrophic fungi in this study. The relative abundance of soil saprotrophic fungi significantly increased from 34% in young forests to 57% in mature forests (Figure 3c), while the EcM fungi decreased from young to mature forests (Figure 3a), which could be attributed to competition within the fungal community (Verbruggen et al., 2017). On the one hand, the interactions between root microbes and soil microbes can be shaped by the root C channel input (de Kroon et al., 2012; Yang et al., 2021). On the other hand, these patterns are likely to reflect adaptations to changes in the soil stoichiometric N:P ratio (Deng et al., 2016; Kranabetter et al., 2019). EcM larch is deciduous with fast decomposition in the litter, so inorganic N might increase in the soil as the forest ecosystem develops (Appendix S1: Figure S10a). With the availability of inorganic, highly mobile N, the advantage of long-distance foraging sources would decrease. Consequently, trees are likely to rely more on the total root surface area rather than long-distance types to capture N. As a result, the ecological niche of long EcM fungi is substituted by the saprotrophic fungi in roots and soil (Kyaschenko et al., 2017; Leake et al., 2002), since they share similar origins and decomposition functions. Furthermore, soil N accumulation results in a relative shortage of soil P due to the synthesis of phosphatase enzymes (Margalef et al., 2021). Notably, the soil N:P ratio in young, middle-aged, and mature plantations was 1.22, 1.25, and 2.82, respectively. We observed that the fungal genus *Penicillium* significantly increased (Figure 3d), and it is known to be one of the most effective fungi for phosphate-solubilizing processes in soil (Reyes et al., 2002).

The complexity among root- and soil-associated fungi peaked during the middle age of the forest, based on the linkage numbers, degree, and path distance of networks according to the network analysis (Appendix S1: Figure S9a). Twieg et al. (2007) also confirmed that the site-level EcM fungal diversity reached a plateau by the age of 26 years. Moreover, the richness of EcM fungi peaked in middle-aged forests ($p = 0.002$) in our study. These results likely reflect the intensity of trading C for nutrients between fungi and plants. During forest development, the stand basal area rate of change may reach its peak during the intermediate stage (Appendix S1: Figure S13), where trees of moderate size exhibit the highest growth rate (Benito et al., 2014; Coomes & Allen, 2007), likely corresponding to peak nutrient demand. The competition between biotrophic and saprotrophic fungi can have a significant influence on fungal composition (Fernandez et al., 2020), as the interactions between host plant C-driven fungi and soil resource (i.e., N)-related fungi depend on forest development in the larch forest. Overall, these networks suggest a complex regulation involving root-fungi-soil feedback, with important implications for the coupling of C and N cycling.

Implications for understanding diverse nutrient foraging strategies

Our study proposed a possible and novel mechanism by which nutrient acquisition strategies might affect the C sequestration capacity of mature forests. Considerable C was consumed by larger roots and the high-turnover, short-distance exploration types of mycorrhizal fungi, which may result in relatively less C allocated to tissue formation and transfer to the soil. Future research should test this mechanism while considering the differing turnover rates of different exploration types. Furthermore, concurrent changes in forest age and soil N during forest development may alter the competition between root-associated and soil-associated fungi, thus impacting C sequestration. Overall, our analysis of root tips and the soil fungi continuum successfully captured the changes from young and explorative to mature and conservative nutrient foraging strategies in EcM larch forest succession (Figure 1b–d). The multi-scale and tripartite functional constraints among root system architecture, root segment physiology, and mycorrhizal fungal exploration type are linked to variations in the size and age of host trees. The temporal spectrum of the nutrient foraging continuum can be explained by metabolic principles. To better understand the underlying causal mechanisms, further research should examine a wide range of EcM tree species and forest ages. Additionally, the nitrogen concentration in the first-order roots shows a narrow range of variation and may already be saturated, regardless of soil nitrogen manipulations. Therefore, global nitrogen deposition may have less impact on the temperate forest's belowground system than previously expected.

CONCLUSION

Forest age explains more variation in the interaction between tree roots and fungi than soil N fertilization in the larch forest, though this pattern may reflect covariation between stand age and unaccounted site-specific characteristics due to the lack of replication within age classes. The succession and self-adapted process of the root-EcM-fungi continuum shows a strong association with root architecture. Our new holistic framework demonstrated that nutrient acquisition is functionally constrained by trade-offs in root system architecture and root segment metabolic activity, and the complementarity between absorptive RLD and mycorrhizal exploration type. Generally, mycorrhizal root strategies are regulated by multiple combinations of plant C and soil N during forest aging. As forests mature, litter accumulates and soil inorganic N increases, less C is used for N foraging, favoring EcM short-distance types, while more C is allocated to maintain the metabolism of the larger root biomass and physiology.

AUTHOR CONTRIBUTIONS

Zeqing Ma developed the overall conceptual framework. Zeqing Ma and Gaigai Ding designed the research. Gaigai Ding performed the experiments and collected data, and Tao Yan provided leaf litter data. Zeqing Ma and Gaigai Ding conducted the analyses and wrote the first draft. Wenjing Zeng, Tao Yan, Lijuan Sun, Weile Chen, and Mingzhen Lu substantially contributed to the revision. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The Illumina MiSeq sequence data generated in this study have been deposited in the National Center for Biotechnology Information (2025) Sequence Read Archive under accession number PRJNA1310041 at http://www.ncbi.nlm.nih.gov/bioproject/1310041. Data and code (Ding et al., 2025) are available in Figshare at https://doi.org/10.6084/m9.figshare.29967025.v1.

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REFERENCES

Agerer, R. 2001. "Exploration Types of Ectomycorrhizae - A Proposal to Classify Ectomycorrhizal Mycelial Systems According to Their Patterns of Differentiation and Putative Ecological Importance." *Mycorrhiza* 11: 107–114.

Ainsworth, E. A., and K. M. Gillespie. 2007. "Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin-Ciocalteu Reagent." *Nature Protocols* 2: 875–77.

Albornoz, F., and J. H. Lieth. 2015. "Diurnal Macronutrients Uptake Patterns by Lettuce Roots Under Various Light and Temperature Levels." *Journal of Plant Nutrition* 38: 2028–43.

Anthony, M. A., T. W. Crowther, S. van der Linde, L. M. Suz, M. I. Bidartondo, F. Cox, M. Schaub, et al. 2022. "Forest Tree Growth Is Linked to Mycorrhizal Fungal Composition and Function Across Europe." *ISME Journal* 16: 1327–36.

Bardgett, R. D., L. Mommer, and F. T. De Vries. 2014. "Going Underground: Root Traits as Drivers of Ecosystem Processes." *Trends in Ecology & Evolution* 29: 692–99.

Bellis, T. D., I. Laforest-Lapointe, K. A. Solarik, D. Gravel, and S. T. Kembel. 2022. "Regional Variation Drives Differences in Microbial Communities Associated with Sugar Maple Across A Latitudinal Range." *Ecology* 103: e3727.

Benito, P. R., J. M. González, S. Ratcliffe, D. A. Coomes, G. Kändler, A. Lehtonen, C. Wirth, and M. A. Zavala. 2014. "Stand Structure and Recent Climate Change Constrain Stand Basal Area Change in European Forests: A Comparison Across Boreal, Temperate, and Mediterranean Biomes." *Ecosystems* 14: 1439–54.

Bergmann, J., A. Weigelt, F. van Der Plas, D. C. Laughlin, T. W. Kuyper, N. R. Guerrero-Ramirez, O. J. Valverde-Barrantes, et al. 2020. "The Fungal Collaboration Gradient Dominates the Root Economics Space in Plants." *Science Advances* 6: aba3756.

Borja, I., H. A. De Wit, A. Steffenrem, and H. Majdi. 2008. "Stand Age and Fine Root Biomass, Distribution and Morphology in a Norway Spruce Chronosequence in Southeast Norway." *Tree Physiology* 28: 773–784.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. "Toward a Metabolic Theory of Ecology." *Ecology* 85: 1771–89.

Brundrett, M. C., and L. Tedersoo. 2018. "Evolutionary History of Mycorrhizal Symbioses and Global Host Plant Diversity." *New Phytologist* 220: 1108–15.

Burton, A. J., J. C. Jarvey, M. P. Jarvi, D. R. Zak, and K. S. Pregitzer. 2011. "Chronic N Deposition Alters Root Respiration-Tissue N Relationship in Northern Hardwood Forests." *Global Change Biology* 18: 258–266.

Carbone, M. S., C. I. Czimczik, T. F. Keenan, P. F. Murakami, N. Pederson, P. G. Schaberg, X. M. Xu, and A. D. Richardson. 2013. "Age, Allocation and Availability of Nonstructural Carbon in Mature Red Maple Trees." *New Phytologist* 200: 1145–55.

Ceccon, C., M. Tagliavini, A. O. Schmitt, and D. M. Eissenstat. 2016. "Untangling the Effects of Root Age and Tissue Nitrogen on Root Respiration in *Populus tremuloides* at Different Nitrogen Supply." *Tree Physiology* 36: 618–627.

Cevallos, E., L. Correa, P. Landázuri, J. Gía, S. Ulloa, D. Rueda, B. Manjunatha, and M. Selvanayagam. 2015. "Evaluate the Effect of Three Levels pH in Leaching and Volatilization of Nitrogen Fertilizers, in Three Soil Types." *Der Pharma Chemica* 7: 521–532.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. "Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor." *Bioinformatics* 34: i884–i890.

Chen, W. L., R. T. Koide, and D. M. Eissenstat. 2018. "Nutrient Foraging by Mycorrhizas: From Species Functional Traits to Ecosystem Processes." *Functional Ecology* 32: 858–869.

Chen, W. L., R. T. Koide, T. S. Adams, J. L. DeForest, L. Cheng, and D. M. Eissenstat. 2016. "Root Morphology and Mycorrhizal Symbioses Together Shape Nutrient Foraging Strategies of Temperate Trees." *Proceedings of the National Academy of Sciences of the United States of America* 113: 8741–46.

Clemmensen, K. E., R. D. Finlay, A. Dahlberg, J. Stenlid, D. A. Wardle, and B. D. Lindahl. 2015. "Carbon Sequestration Is Related to Mycorrhizal Fungal Community Shifts During Long-Term Succession in Boreal Forests." *New Phytologist* 205: 1525–36.

Comas, L. H., T. J. Bouma, and D. M. Eissenstat. 2002. "Linking Root Traits to Potential Growth Rate in Six Temperate Tree Species." *Oecologia* 132: 34–43.

Coomes, D. A., and R. B. Allen. 2007. "Effects of Size, Competition and Altitude on Tree Growth." *Journal of Ecology* 95: 1084–97.

de Kroon, H., M. Hendriks, J. van Ruijven, J. Ravenek, F. M. Padilla, E. Jongejans, E. J. W. Visser, and L. Mommer. 2012. "Root Responses to Nutrients and Soil Biota: Drivers of Species Coexistence and Ecosystem Productivity." *Journal of Ecology* 100: 6–15.

Deng, M., L. Liu, Z. Sun, S. Piao, Y. Ma, Y. Chen, J. Wang, C. Qiao, X. Wang, and P. Li. 2016. "Increased Phosphate Uptake but Not Resorption Alleviates Phosphorus Deficiency Induced by Nitrogen Deposition in Temperate *Larix principis-rupprechtii* Plantations." *New Phytologist* 212: 1019–29.

Ding, G. G., W. J. Zeng, T. Yan, L. J. Sun, W. L. Chen, M. Z. Lu, and Z. Q. Ma. 2025. "Data and Code for Main Results of 'Root-Mycorrhizal Foraging Strategies Shift with Forest Age More than with Nitrogen manipulation'." Figshare. Online Resource. https://doi.org/10.6084/m9.figshare.29967025.v1

Edgar, R. C. 2013. "UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads." *Nature Methods* 10: 996–98.

Eissenstat, D. M. 1991. "On the Relationship Between Specific Root Length and the Rate of Root Proliferation -A Field-Study Using Citrus Rootstocks." *New Phytologist* 118: 63–68.

Eissenstat, D. M., and R. D. Yanai. 1997. "The Ecology of Root Lifespan." *Advances in Ecological Research* 27: 1–60.

Eissenstat, D. M., J. M. Kucharski, M. Zadworny, T. S. Adams, and R. T. Koide. 2015. "Linking Root Traits to Nutrient Foraging in Arbuscular Mycorrhizal Trees in a Temperate Forest." *New Phytologist* 208: 114–124.

Faust, K. 2021. "Open Challenges for Microbial Network Construction and Analysis." *The ISME Journal* 15: 3111–18.

Fernandez, C. W., and P. G. Kennedy. 2016. "Revisiting the 'Gadgil Effect': Do Interguild Fungal Interactions Control Carbon Cycling in Forest Soils?" *New Phytologist* 209: 1382–94.

Fernandez, C. W., C. R. See, and P. G. Kennedy. 2020. "Decelerated Carbon Cycling by Ectomycorrhizal Fungi Is Controlled by Substrate Quality and Community Composition." *New Phytologist* 226: 569–582.

Fitter, A. H. 1987. "An Architectural Approach to the Comparative Ecology of Plant Root Systems." *New Phytologist* 106: 61–77.

Freschet, G. T., E. M. Swart, and J. H. C. Cornelissen. 2015. "Integrated Plant Phenotypic Responses to Contrasting Above- and Below-Ground Resources: Key Roles of Specific Leaf Area and Root Mass Fraction." *New Phytologist* 206: 1247–60.

Gauthier, S., P. Bernier, T. Kuuluvainen, A. Z. Shvidenko, and D. G. Schepaschenko. 2015. "Boreal Forest Health and Global Change." *Science* 349: 819–822.

Geml, J. 2019. "Soil Fungal Communities Reflect Aspect-Driven Environmental Structuring and Vegetation Types in A Pannonian Forest Landscape." *Fungal Ecology* 39: 63–79.

Geml, J., L. N. Morgado, T. A. Semenova-Nelsen, and M. Schilthuizen. 2017. "Changes in Richness and Community Composition of Ectomycorrhizal Fungi Among Altitudinal Vegetation Types on Mount Kinabalu in Borneo." *New Phytologist* 215: 454–468.

Gong, J. R., Z. W. Ge, R. An, Q. W. Duan, X. You, and Y. M. Huang. 2012. "Soil Respiration in Poplar Plantations in Northern China at Different Forest Ages." *Plant and Soil* 360: 109–122.

Guo, D., M. Xia, X. Wei, W. Chang, Y. Liu, and Z. Wang. 2008. "Anatomical Traits Associated with Absorption and Mycorrhizal Colonization Are Linked to Root Branch Order in Twenty-Three Chinese Temperate Tree Species." *New Phytologist* 180: 673–683.

Guo, M. S., G. L. Gao, G. D. Ding, and Y. Zhang. 2020. "Drivers of Ectomycorrhizal Fungal Community Structure Associated with *Pinus sylvestris var. Mongolica* Differ at Regional vs. Local Spatial Scales in Northern China." *Forests* 11: 323.

Hagenbo, A., J. Kyaschenko, K. E. Clemmensen, B. D. Lindahl, and P. Fransson. 2018. "Fungal Community Shifts Underpin Declining Mycelial Production and Turnover Across a *Pinus sylvestris* Chronosequence." *Journal of Ecology* 106: 490–501.

Hasselquist, N. J., and P. Högberg. 2014. "Dosage and Duration Effects of Nitrogen Additions on Ectomycorrhizal Sporocarp Production and Functioning: An Example from Two N-Limited Boreal Forests." *Ecology and Evolution* 4: 3015–26.

Hobbie, E. A. 2006. "Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies." *Ecology* 87: 563–69.

Hobbie, J. E., and E. A. Hobbie. 2006. "^{15}N in Symbiotic Fungi and Plants Estimates Nitrogen and Carbon Flux Rates in Arctic Tundra." *Ecology* 87: 816–822.

Hodge, A. 2004. "The Plastic Plant: Root Responses to Heterogeneous Supplies of Nutrients." *New Phytologist* 162: 9–24.

Hodge, A., and K. Storer. 2015. "Arbuscular Mycorrhiza and Nitrogen: Implications for Individual Plants Through to Ecosystems." *Plant and Soil* 386: 1–19.

Jiao, S., W. Chen, J. Wang, N. Du, Q. Li, and G. Wei. 2018. "Soil Microbiomes with Distinct Assemblies Through Vertical Soil Profiles Drive the Cycling of Multiple Nutrients in Reforested Ecosystems." *Microbiome* 6: 1–16.

Jobbagy, E. G., and R. B. Jackson. 2004. "The Uplift of Soil Nutrients by Plants: Biogeochemical Consequences Across Scales." *Ecology* 85: 2380–89.

Jörgensen, K., G. Granath, J. Strengbom, and B. D. Lindahl. 2021. "Links Between Boreal Forest Management, Soil Fungal Communities and Below-Ground Carbon Sequestration." *Functional Ecology* 36: 392–405.

Jörgensen, K., K. E. Clemmensen, H. Wallander, and B. D. Lindahl. 2022. "Do Ectomycorrhizal Exploration Types Reflect Mycelial Foraging Strategies?" *New Phytologist* 237: 576–584.

Kodama, T., T. Ichikawa, K. Hidaka, and K. Furuya. 2014. "A Highly Sensitive and Large Concentration Range Colorimetric Continuous Flow Analysis for Ammonium Concentration." *Journal of Oceanography* 71: 65–75.

Koljalg, U., K. H. Larsson, K. Abarenkov, R. H. Nilsson, I. J. Alexander, U. Eberhardt, S. Erland, et al. 2005. "UNITE: A Database Providing Web-Based Methods for the Molecular Identification of Ectomycorrhizal Fungi." *New Phytologist* 166: 1063–68.

Kong, D. L., C. G. Ma, Q. Zhang, L. Li, X. Y. Chen, H. Zeng, and D. L. Guo. 2014. "Leading Dimensions in Absorptive Root Trait Variation Across 96 Subtropical Forest Species." *New Phytologist* 203: 863–872.

Kranabetter, J. M., R. Harman-Denhoed, and B. J. Hawkins. 2019. "Saprotrophic and Ectomycorrhizal Fungal Sporocarp Stoichiometry (C:N:P) Across Temperate Rainforests as Evidence of Shared Nutrient Constraints among Symbionts." *New Phytologist* 221: 482–492.

Kyaschenko, J., K. E. Clemmensen, A. Hagenbo, E. Karltun, and B. D. Lindahl. 2017. "Shift in Fungal Communities and Associated Enzyme Activities Along an Age Gradient of Managed *Pinus sylvestris* Stands." *The ISME Journal* 11: 863–874.

Lajoie, G., and S. T. Kembel. 2021. "Host Neighborhood Shapes Bacterial Community Assembly and Specialization on Tree Species Across A Latitudinal Gradient." *Ecological Monographs* 91: e01443.

Leake, J. R., D. P. Donnelly, and L. Boddy. 2002. "Interactions Between Ecto-Mycorrhizal and Saprotrophic Fungi." *Mycorrhizal ecology* 157: 345–372.

Leduc, S. D., E. A. Lilleskov, T. R. Horton, and D. E. Rothstein. 2013. "Ectomycorrhizal Fungal Succession Coincides with Shifts in Organic Nitrogen Availability and Canopy Closure in Post-Wildfire Jack Pine Forests." *Oecologia* 172: 257–269.

Lilleskov, E. A., E. A. Hobbie, and T. R. Horton. 2011. "Conservation of Ectomycorrhizal Fungi: Exploring the Linkages between Functional and Taxonomic Responses to Anthropogenic N Deposition." *Fungal Ecology* 4: 174–183.

Lilleskov, E. A., T. D. Bruns, T. R. Horton, D. L. Taylor, and P. Grogan. 2004. "Detection of Forest Stand-Level Spatial Structure in Ectomycorrhizal Fungal Communities." *FEMS Microbiology Ecology* 49: 319–332.

Lindahl, B. D., and A. Tunlid. 2014. "Ectomycorrhizal Fungi-Potential Organic Matter Decomposers, Yet Not Saprotrophs." *New Phytologist* 205: 1443–47.

Liu, B. T., H. B. Li, B. A. Zhu, R. T. Koide, D. M. Eissenstat, and D. L. Guo. 2015. "Complementarity in Nutrient Foraging Strategies of Absorptive Fine Roots and Arbuscular Mycorrhizal Fungi Across 14 Coexisting Subtropical Tree Species." *New Phytologist* 208: 125–136.

Luyssaert, S., E. D. Schulze, A. Borner, A. Knohl, D. Hessenmoller, B. E. Law, P. Ciais, and J. Grace. 2008. "Old-Growth Forests as Global Carbon Sinks." *Nature* 455: 213–15.

Ma, Z. Q., D. L. Guo, X. L. Xu, M. Z. Lu, R. D. Bardgett, D. M. Eissenstat, M. L. McCormack, and L. O. Hedin. 2018. "Evolutionary History Resolves Global Organization of Root Functional Traits." *Nature* 555: 94–97.

Maaroufi, N. I., A. Nordin, K. Palmqvist, N. J. Hasselquist, B. Forsmark, N. P. Rosenstock, H. Wallander, and M. J. Gundale. 2019. "Anthropogenic Nitrogen Enrichment Enhances Soil Carbon Accumulation by Impacting Saprotrophs Rather than Ectomycorrhizal Fungal Activity." *Global Change Biology* 25: 2900–2914.

Magoc, T., and S. L. Salzberg. 2011. "FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies." *Bioinformatics* 27: 2957–63.

Makkonen, K., and H. S. Helmisaari. 2001. "Fine Root Biomass and Production in Scots Pine Stands in Relation to Stand Age." *Tree Physiology* 21: 193–98.

Margalef, O., J. Sardans, J. Maspons, R. Molowny-Horas, M. Fernandez-Martinez, I. A. Janssens, A. Richter, P. Ciais, M. Obersteiner, and J. Penuelas. 2021. "The Effect of Global Change on Soil Phosphatase Activity." *Global Change Biology* 27: 5989–6003.

Martin-Pinto, P., J. A. Oria-de-Rueda, T. Dejene, O. Mediavilla, M. Hernandez-Rodriguez, J. A. Reque, I. Sanz-Benito, M. Santos, and J. Geml. 2022. "Influence of Stand Age and Site Conditions on Ectomycorrhizal Fungal Dynamics in *Cistus ladanifer*-Dominated Scrubland Ecosystems." *Forest Ecology and Management* 519: 120340.

McConnaughay, K., and F. J. F. E. Bazzaz. 1992. "The Occupation and Fragmentation of Space: Consequences of Neighbouring Roots." *Functional Ecology* 6: 704–710.

McGeehan, S. L., and D. V. Naylor. 2008. "Automated Instrumental Analysis of Carbon and Nitrogen in Plant and Soil Samples." *Communications in Soil Science and Plant Analysis* 19: 493–505.

McNickle, G. G., M. K. Deyholos, J. F. Cahill, and J. Schweitzer. 2015. "Nutrient Foraging Behaviour of Four Co-Occurring Perennial Grassland Plant Species Alone Does Not Predict Behaviour with Neighbours." *Functional Ecology* 30: 420–430.

Mendes, L. W., J. M. Raaijmakers, M. de Hollander, R. Mendes, and S. M. Tsai. 2018. "Influence of Resistance Breeding in Common Bean on Rhizosphere Microbiome Composition and Function." *ISME Journal* 12: 212–224.

Nakamura, T., and M. Nakamura. 2016. "Root Respiratory Costs of Ion Uptake, Root Growth, and Root Maintenance in Wetland Plants: Efficiency and Strategy of O_2 Use for Adaptation to Hypoxia." *Oecologia* 182: 667–678.

National Center for Biotechnology Information. 2025. "Sequence Read Archive Accession No. PRJNA1310041." www.ncbi.nlm.nih.gov/bioproject/1310041.

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling, and P. G. Kennedy. 2016. "FUNGuild: An Open Annotation Tool for Parsing Fungal Community Datasets by Ecological Guild." *Fungal Ecology* 20: 241–48.

Niklas, K. J. 1994. *Plant Allometry: The Scaling of Form and Process*. Chicago, IL: University of Chicago Press.

Niklas, K. J., and B. J. Enquist. 2001. "Invariant Scaling Relationships for Interspecific Plant Biomass Production Rates and Body Size." *Proceedings of the National Academy of Sciences of the United States of America* 98: 2922–27.

Noguchi, K., J. Nagakura, and S. Kaneko. 2013. "Biomass and Morphology of Fine Roots of Sugi (Cryptomeria Japonica) after 3 Years of Nitrogen Fertilization." *Frontiers in Plant Science* 4: 347.

Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2011. "Rethinking Ectomycorrhizal Succession: Are Root Density and Hyphal Exploration Types Drivers of Spatial and Temporal Zonation?" *Fungal Ecology* 4: 233–240.

Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. "The Mycorrhizal-Associated Nutrient Economy: A New Framework for Predicting Carbon-Nutrient Couplings in Temperate Forests." *New Phytologist* 199: 41–51.

Põlme, S., K. Abarenkov, R. Henrik Nilsson, B. D. Lindahl, K. E. Clemmensen, H. Kauserud, N. Nguyen, et al. 2021. "Fungaltraits: A User-Friendly Traits Database of Fungi and Fungus-like Stramenopiles." *Fungal Diversity* 105: 1–16.

Poorter, H., K. J. Niklas, P. B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. "Biomass Allocation to Leaves, Stems and Roots: Meta-Analyses of Interspecific Variation and Environmental Control." *New Phytologist* 193: 30–50.

Pregitzer, K., J. Deforest, A. J. Burton, M. F. Allen, R. W. Ruess, and R. Hendrick. 2002. "Fine Root Architecture of Nine North American Trees." *Ecological Monographs* 72: 293–309.

Qu, T., T. Yan, W. Zhang, and H. Zeng. 2019. "Responses of Herbaceous Community Characteristics and Biomass to Nitrogen Addition in A *Larix principis-rupprechtii* Plantation." *Acta Scientiarum Naturalium Universitatis Pekinensis* 55: 587–596.

Raich, J. W., D. A. Clark, L. Schwendenmann, and T. E. Wood. 2014. "Aboveground Tree Growth Varies with Belowground Carbon Allocation in A Tropical Rainforest Environment." *PLoS One* 9: e100275.

Reich, P. B., M. B. Walters, M. G. Tjoelker, D. Vanderklein, and C. Buschena. 1998. "Photosynthesis and Respiration Rates Depend on Leaf and Root Morphology and Nitrogen Concentration in Nine Boreal Tree Species Differing in Relative Growth Rate." *Functional Ecology* 12: 395–405.

Ren, H., G. Gao, Y. Ma, Z. Li, S. Wang, J. Gu, and E. Inselsbacher. 2023. "Shift of Root Nitrogen-Acquisition Strategy with Tree Age Is Mediated by Root Functional Traits along the Collaboration Gradient of the Root Economics Space." *Tree Physiology* 43: 1341–53.

Reyes, I., L. Bernier, and H. Antoun. 2002. "Rock Phosphate Solubilization and Colonization of Maize Rhizosphere by Wild and Genetically Modified Strains of *Penicillium rugulosum*." *Microbial Ecology* 44: 39–48.

Robinson, D., A. Hodge, B. S. Griffiths, and A. H. Fitter. 1999. "Plant Root Proliferation in Nitrogen-Rich Patches Confers Competitive Advantage." *Proceedings of the Royal Society of London. Series B, Biological Sciences* 266: 431–35.

Rosenvald, K., I. Ostonen, V. Uri, M. Varik, L. Tedersoo, and K. Lohmus. 2013. "Tree Age Effect on Fine-Root and Leaf Morphology in A Silver Birch Forest Chronosequence." *European Journal of Forest Research* 132: 219–230.

Rudawska, M., R. Wilgan, D. Janowski, M. Iwanski, and T. Leski. 2018. "Shifts in Taxonomical and Functional Structure of Ectomycorrhizal Fungal Community of Scots Pine (*Pinus sylvestris* L.) Underpinned by Partner Tree Ageing." *Pedobiologia* 71: 20–30.

Ryser, P., and H. Lambers. 1995. "Root and Leaf Attributes Accounting for the Performance of Fast-Growing and Slow-Growing Grasses at Different Nutrient Supply." *Plant and Soil* 170: 251–265.

Shakhmatov, R., S. Hashiguchi, T. C. Maximov, and A. Sugimoto. 2022. "Effects of Snow Manipulation on Larch Trees in the Taiga Forest Ecosystem in Northeastern Siberia." *Progress in Earth and Planetary Science* 9: 1–16.

Steidinger, B. S., T. W. Crowther, J. Liang, M. E. Van Nuland, G. D. A. Werner, P. B. Reich, G. J. Nabuurs, et al. 2019. "Climatic Controls of Decomposition Drive the Global Biogeography of Forest-Tree Symbioses." *Nature* 569: 404–8.

Sun, Z., L. Liu, S. Peng, J. Peñuelas, H. Zeng, and S. Piao. 2016. "Age-Related Modulation of the Nitrogen Resorption Efficiency Response to Growth Requirements and Soil Nitrogen Availability in a Temperate Pine Plantation." *Ecosystems* 19: 698–709.

Tamooh, F., M. Huxham, M. Karachi, M. Mencuccini, J. G. Kairo, and B. Kirui. 2008. "Below-Ground Root Yield and Distribution in Natural and Replanted Mangrove Forests at Gazi Bay, Kenya." *Forest Ecology and Management* 256: 1290–97.

Tedersoo, L., and M. E. Smith. 2013. "Lineages of Ectomycorrhizal Fungi Revisited: Foraging Strategies and Novel Lineages Revealed by Sequences from Belowground." *Fungal Biology Reviews* 27: 83–99.

Tedersoo, L., T. Naadel, M. Bahram, K. Pritsch, F. Buegger, M. Leal, U. Koljalg, and K. Poldmaa. 2012. "Enzymatic Activities and Stable Isotope Patterns of Ectomycorrhizal Fungi in Relation to Phylogeny and Exploration Types in An Afrotropical Rain Forest." *New Phytologist* 195: 832–843.

Terrer, C., R. P. Phillips, B. A. Hungate, J. Rosende, J. Pett-Ridge, M. E. Craig, K. J. van Groenigen, et al. 2021. "A Trade-Off between Plant and Soil Carbon Storage under Elevated CO_2." *Nature* 591: 599–603.

Tiyapongpattana, W., P. Pongsakul, J. Shiowatana, and D. Nacapricha. 2004. "Sequential Extraction of Phosphorus in Soil and Sediment Using a Continuous-Flow System." *Talanta* 62: 765–771.

Twieg, B. D., D. M. Durall, and S. W. Simard. 2007. "Ectomycorrhizal Fungal Succession in Mixed Temperate Forests." *New Phytologist* 176: 437–447.

Verbruggen, E., R. Pena, C. W. Fernandez, and J. L. Soong. 2017. "Mycorrhizal Interactions with Saprotrophs and Impact on Soil Carbon Storage." In *Mycorrhizal Mediation of Soil*, 441–460. Amsterdam: Elsevier.

Wahl, S., and P. Ryser. 2000. "Root Tissue Structure Is Linked to Ecological Strategies of Grasses." *New Phytologist* 148: 459–471.

Wang, G., G. Lin, Y. Zhang, L. Zheng, D. H. Zeng, and H. Lambers. 2024. "Shifts from an Extensive to an Intensive Root Nutrient-Acquisition Mode with Stand Development of Three Pinus Species." *Journal of Ecology* 112: 886–900.

Wasyliw, J., and J. Karst. 2020. "Shifts in Ectomycorrhizal Exploration Types Parallel Leaf and Fine Root Area with Forest Age." *Journal of Ecology* 108: 2270–82.

Weemstra, M., F. J. Sterck, E. J. W. Visser, T. W. Kuyper, L. Goudzwaard, and L. Mommer. 2017. "Fine-Root Trait Plasticity of Beech (*Fagus sylvatica*) and Spruce (*Picea abies*) Forests on Two Contrasting Soils." *Plant and Soil* 415: 175–188.

Weiner, J. 2004. "Allocation, Plasticity and Allometry in Plants." *Perspectives in Plant Ecology Evolution and Systematics* 6: 207–215.

Wen, T., P. Xie, S. Yang, G. Niu, X. Liu, Z. Ding, C. Xue, Y. X. Liu, Q. Shen, and J. Yuan. 2022. "ggClusterNet: An R Package for Microbiome Network Analysis and Modularity-Based Multiple Network Layouts." *iMeta* 1: e32.

West, G. B., J. H. Brown, and B. J. Enquist. 1997. "A General Model for the Origin of Allometric Scaling Laws in Biology." *Science* 276: 122–26.

West, G. B., J. H. Brown, and B. J. Enquist. 1999. "A General Model for the Structure and Allometry of Plant Vascular Systems." *Nature* 400: 664–67.

Yan, T., T. Qu, Z. Sun, R. Dybzinski, A. Chen, X. Yao, H. Zeng, and S. Piao. 2018. "Negative Effect of Nitrogen Addition on Soil Respiration Dependent on Stand Age: Evidence from a 7-Year Field Study of Larch Plantations in Northern China." *Agricultural and Forest Meteorology* 262: 24–33.

Yang, T., L. Tedersoo, X. Fu, C. Zhao, X. Liu, G. Gao, L. Cheng, J. M. Adams, and H. Chu. 2021. "Saprotrophic Fungal Diversity Predicts Ectomycorrhizal Fungal Diversity along the Timberline in the Framework of Island Biogeography Theory." *ISME Communications* 1: 15.

Yuan, Z. Y., and H. Y. H. Chen. 2012. "Fine Root Dynamics with Stand Development in the Boreal Forest." *Functional Ecology* 26: 991–98.

Zhao, X., Q. Tian, L. Huang, Q. Lin, J. Wu, and F. Liu. 2022. "Fine-Root Functional Trait Response to Nitrogen Deposition Across Forest Ecosystems: A Meta-Analysis." *Science of the Total Environment* 844: 157111.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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