Climate-induced shifts in ectomycorrhizal explorations from long to short strategies along an elevation gradient

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Summary

- Ectomycorrhizal (EcM) fungi colonize over 60% of tree stems globally, yet how their hyphal morphology varies across environments remains unclear, and current methods for defining EcM mycelial morphology and foraging space lack reliability.
- Here, we analyzed the morphological traits of 53,262 EcM root tips of *Abies fargesii* var. *faxoniana* across elevational temperature gradients (0.2–5.1°C) and developed a species-morphotype-space (SMS) framework to quantify mycelial foraging strategies from root tips to the soil profile.
- Our results revealed considerable EcM morphotype complexity at the root tip level and distinct temperature- and soil nutrient-driven shifts in EcM morphology based on an elevational gradient. Short-hyphae dominated at lower elevation, whereas thicker long-rhizomorphs prevailed at higher elevation, reaching a foraging space of up to 1.02×10^{6} cm^{3} m^{-3} soil. Short-hyphae mycorrhizae exhibited high active hydrolytic enzymes, which enable them to elevate resource availability and play a pivotal role in nutrient foraging. As temperatures rise, increased soil inorganic nitrogen availability in cold forests favors short-hyphae types while suppressing long-rhizomorph types with high carbon retention capacity, potentially reducing soil carbon stability.
- Our analyses of EcM foraging space and multi-dimensional mycelial diversity provide new opportunities to predict how EcM hyphal processes respond to climate warming.

Keywords: ectomycorrhizal morphology, ectomycorrhizal taxonomic diversity, hyphal exploration types, phenotypic structure, foraging function

Introduction

Ectomycorrhizal (EcM) fungi colonize 2% of tree species, but they form symbiosis with over 60% of tree stems globally (Brundrett & Tedersoo, 2018; Steidinger *et al.*, 2019). EcM fungi also exhibit enormous morphological variation across an estimated ~20,000 species (Agerer, 2006; Brundrett, 2009; Tedersoo & Smith, 2013), and play key roles in plant resource acquisition (Agerer *et al.*, 2012; Pellitier & Zak, 2021), soil carbon (C) sequestration (Terrer *et al.*, 2021), and nutrient cycling (Suz *et al.*, 2021). However, despite the known importance of EcM for ecosystem functioning, it remains unclear how the morphology of EcM mycelium varies across ecosystems and with climate change (Agerer, 2006; van der Linde *et al.*, 2018; Jörgensen *et al.*, 2023). This represents an important knowledge gap given the importance of EcM mycelium morphology for nutrient foraging, a key regulator of biogeochemical cycles of EcM-rich forest ecosystems. As such, accurate quantification of EcM mycelium morphology could enable improved understanding and prediction of the response of EcM hyphal-driven processes to climate change (Jörgensen *et al.*, 2023).

A key feature of EcM mycelium morphology is the existence of different hyphal exploration types, which differ in their strategies of resource foraging (Agerer, 2001). Notably, long-distance EcM taxa form rhizomorphs and have a slow turnover, creating hyphal networks to access spatially scattered resources (Cairney, 2005; Hobbie & Agerer, 2010); whereas short-distance EcM hyphae cycle rapidly, enabling them to rapidly exploit nutrient-rich patches (Hobbie & Agerer, 2010; Bahr *et al.*, 2015). It has also been proposed that the adaptive responses of EcM fungi to different environments vary between short and long exploration types, and correspond to species composition and morphology. In cold environments, such as the Arctic, long-distance rhizomorph forming EcM fungi (i.e., *Xerocomus badius*) tend to have a competitive advantage due to their extra ability to capture organic nitrogen (Clemmensen *et al.*, 2015; Morgado *et al.*, 2015); whereas in warmer regions, EcM fungi with short-distance hyphae are more prevalent (Fernandez *et al.*, 2017), as tree species may allocate more resources to fungal taxa with shorter hyphae that have greater reproductive and dispersal capabilities, rather than investing in extraradical mycelium under warming conditions (Cripps, 2004; Nara & Hogetsu, 2004). There is also evidence that warming favors fungi specialized in short-distance type in EcM-dominated and cold environments (Fernandez *et al.*, 2017; Steidinger *et al.*, 2019; Fernandez *et al.*, 2023). Whether rising temperatures promote short-distance hyphae while inhibiting long-distance hyphae, however, remains untested in natural ecosystems or controlled experiments (Jarvis *et al.*, 2013; Fernandez *et al.*, 2017; Defrenne *et al.*, 2019; Parts *et al.*, 2019), despite the well-known sensitivity of EcM in cold, high-altitude regions to climate change (Pickles *et al.*, 2012; Miyamoto *et al.*, 2015).

Another central but unresolved issue is whether EcM fungal species detected by sequencing are linked to hyphal exploration types as identified through morphological examination (Tedersoo & Smith, 2013; Jörgensen *et al.*, 2023). If they are, sequencing and morphological traits can be unified along a single dimension to predict the foraging function. Alternatively, if morphological characteristics or hyphal exploration types are independently expressed, it would suggest that the EcM organization may be more complex. This complexity may arise, in part, from interactions between EcM morphological traits and various factors, such as host plant root traits (Peay *et al.*, 2011; Defrenne *et al.*, 2019), soil resources (Pellitier & Zak, 2021; Jörgensen *et al.*, 2023), environmental conditions (Smith & Read, 2010; Jarvis *et al.*, 2013), and biotic factors (van der Linde *et al.*, 2018). Indeed, it has been demonstrated through the use of ingrowth mesh bags that hyphal foraging strategies cannot be predicted by soil exploration types inferred from EcM species information (Jörgensen *et al.* 2023), and high morphological plasticity of EcM species has been documented across environmental gradients (van der Linde *et al.* (2018). Consequently, EcM species identity may be an unreliable predictor of hyphal morphological foraging strategies at root-tip in changing environments, highlighting a critical gap that requires validation through empirically measured hyphal foraging functional traits (e.g. hyphal length, diameter, etc.).

Furthermore, comparative data on foraging space—mycelium spatial structure is scarce due to the spatial heterogeneity of mycelium (Weigt *et al.*, 2012), the complex hyphal dynamics expressed by meta-species populations (Agerer *et al.*, 2012), and the laborious nature of measuring. EcM hyphae can extend up to 42 cm in length from the root surface (Schramm, 1965; Agerer, 2001), have a high turnover rate (Ekblad *et al.*, 2013; Clemmensen *et al.*, 2015), and produce enzymes to explore soil patches and forage for nutrients (Tedersoo *et al.*, 2012). Their morphological traits might be a key dimension for evaluating their absorptive functions (Zhu *et al.*, 2023), construction cost (Corrêa & Martins-Loução, 2011), and C storage (See *et al.*, 2022). However, accurately capturing the 3D organization of hyphae (Agerer, 2001; See *et al.*, 2022) remains challenging, which limits our ability to predict EcM foraging processes and their response to climate change.

Here, we explored how key EcM morphological traits respond to temperature change based on the analysis of EcM mycelia of tree root tips of *Abies fargesii* var. *faxoniana* (Faxon fir), the dominant tree species along an elevation gradient that included five sites in the Wolong Nature Reserve, China. We also introduce a new 'species-morphotype-space' (SMS) framework to define the spatial structure of EcM mycelia from the root to the soil profile scale and disentangle responses to temperature change. It is well established that declining temperature with increasing elevation is a major driver of plant communities and ecosystem dynamics (Körner, 1998; Mayor *et al.*, 2017), and that elevational gradients, as thermoclines, enable prediction of long-term ecological responses to climate change, acting as a proxy for temperature change and future warming (Körner, 1998). Specifically, we examined 53, 262 EcM root tips along the elevation gradient, measured EcM morphological traits by using microscopy, and then sequenced the EcM morphotypes that we identified. We also estimated the foraging space occupied by mycelia, mathematically scaling hyphae space to the soil profile level. Thus, we created a unique comprehensive dataset that included 26 root-mycorrhizal functional traits and nitrogen uptake rates (NUR), and a wide range of corresponding soil and climatic factors (Extended Data Table 1).

We hypothesised that: (i) short-distance fungi decrease with increasing elevation, but increase with increasing temperature; (ii) EcM exploration types identified by morphological traits are inconsistent with those inferred from fungal species (OTUs); and (iii) responses of foraging space to temperature change among different exploration types, directly quantified by morphological traits, are less linked to fungal species composition (OTUs).

Methods

Experimental design and investigation.

A field experiment was conducted to investigate the relationships between the structure and function of EcM fungi and their response to temperature changes. Increasing elevation and decreasing temperatures are major drivers of plant, EcM fungi community composition, and foraging space (Körner, 1998; Mayor *et al.*, 2017), particularly within regions with similar evolutionary backgrounds. Thus, we selected five elevational gradients, which act as natural thermoclines, to provide a valuable proxy for predicting long-term EcM responses to climate change (Körner, 1998). We controlled for the host plant by selecting *Abies fargesii* var. *faxoniana* in its pure forest habitat as the study subject. This species is widely distributed in the subalpine region along the eastern flanks of the Tibetan Plateau, at elevations ranging from 2600 to 3900 m asl, where it experiences a broad range of environmental gradients, from subtropical semi-humid to cold, high-mountain climates (Li *et al.*, 2020). This site is located in the Wolong Nature Reserve (30°51′00″ N, 102°58′12″ E) in Sichuan Province, China. The region experiences dry, cold winters and wet, cool summers, with a mean annual temperature of 4.06 °C and mean annual precipitation of approximately 1063 mm (Chen *et al.*, 2021). The forest canopy is dominated by *Abies faxoniana* var. *faxoniana*, with a sub-canopy primarily composed of *Rhododendron faberi* Hemsl. and *Rhododendron asterochnoum* Diels.

At each elevation, we selected eight central points within the 100 m belt transect (Mitchell, 2010). At each central point, we established a 10-meter radius sampling circle. We measured tree height, diameter at breast height (DBH), and soil properties at each sampling point (Köhler *et al.*, 2018). For soil sampling, we collected cubic samples 1 meter away from each target tree. A total of 160 soil blocks (10 × 10 × 10 cm^3) (4 target trees × 8 replications × 5 elevations) were collected, with four target trees sampled at each central point. The soil samples were stored in ziplock bags and transferred to a laboratory refrigerator for processing.

The roots of *Abies fargesii* var. *faxoniana* are easily identifiable by their reddish-brown color and distinct aromatic scent. We collected 160 samples of absorptive fine roots (with a diameter <2 mm) from the soil. After separating the roots, we combined four soil samples from each central point, resulting in 40 composite soil samples. We divided the total root samples into two parts: one half ($N = 80$) was used to measure EcM morphotypes and count root tips, with samples stored in 5% glycerin at −20°C (Köhler *et al.*, 2018); the other half ($N = 80$) was prepared for measuring root traits and biomass.

Observation and measurements of EcM morphology.

After cleaning the soil and washing it with water, we cut the root samples into 5 cm segments for observation under a stereo microscope (Leica, M205FA, Germany). We recorded whether the root tip was covered by a fungal mantle, indicating ectomycorrhizae, or not. In total, we observed 53,262 root tips with EcM structures from root samples collected from 80 soil block samples.

We specifically focused on the morphological characteristics of EcM, such as color, mantle surface structure, emanating hyphae, and rhizomorphs. We also examined the microscopic characteristics of the mycorrhizae, including the smoothness of the mantle surface and the pattern of hyphal differentiation at the root tip. These morphological types were identified based on the criteria established by Rambold and

Agerer (1997). We then grouped similar EcM systems into distinct morphological types, following the classifications by Agerer (2001), Agerer (2006), Rambold and Agerer (1997). In total, we identified and classified 51 morphotypes from the 53,262 root tips. The morphological traits of these morphotypes are summarized in Table S2.

During observation, we quantified the mantle diameter (D_{mantle}) and length (L_{mantle}) at the distal root, and hyphae diameter (d_{hyphae}) and maximum hyphae length (L_{hyphae}) for each morphotype (Fig. S1; Fig.1a). These trait values were averaged over three replicates. If fewer than three root tips of the same morphotype were observed, we measured all available samples for that morphotype. To simplify the classification of exploration types, we categorized 51 morphotypes into three hyphal exploration types by the morphologies (*e.g.*, length, diameter, etc.) of the extraradical emanating (Table S2): i) few-hyphae, characterized by little to no emanating hyphae; ii) short-hyphae, defined by short hyphae according to Agerer (2001); iii) long-rhizomorph, characterized by rhizomorphic hyphae, which includes three medium- and one long-distance exploration types as defined by Agerer (2001).

The mycorrhizal colonization ratio was determined by counting the number of root tips of each EcM hyphal exploration type (few-hyphae, short-hyphae, and long-rhizomorph) in each soil block:

$$Colonization\ ratio(\%) = \frac{Counts\ \text{of EcM root tips}}{Counts\ of\ total\ root\ tips} \times 100\% \ \text{[Eq.1]}$$

We then calculated the mycorrhizal colonization ratio for each exploration type based on the recorded root tip counts, and also calculated the proportion of root tips for each morphotype at each elevation relative to the total number of EcM root tips. Finally, samples from each morphotype (N=51) were stored at -80°C for DNA sequencing. The remaining EcM root tips from each mixed soil sample (N=40) were preserved at -80°C to analyze community fungal species diversity.

Estimation of EcM and root foraging space.

EcM fungi form extensive networks of hyphae, which serve as their primary foraging structures, exploring the soil to acquire water and nutrients for their host plant. This foraging space, defined by the mycelium's spatial structure (its extent, density, and 3D architecture) (Agerer, 2001), is a critical parameter of fungal function. To compare the nutrient foraging space among roots and ectomycorrhizae, we first estimated the length, volume, and biomass of fine roots in the soil matrix. Root samples from each soil block (10×10×10 cm^3, N=80) were collected and gently washed to analyze root foraging space. We scanned all the fine roots from each soil block and extracted the total root length and root volume from the scanned images using WinRHIZO 2009 (Regent Instruments, Quebec, QC, Canada). The root length density (L_{root}, cm m^{-3}) was determined as the total root length per cubic meter of soil, while root volume density (V_{root}, m^3 m^{-3}) was estimated as the total root volume per cubic meter of soil. We then measured the total dry weight of the fine roots from each soil block to calculate the root biomass (B_{root}, g m^{-3}).

We developed a novel method to scale EcM morphological traits from single root rips to soil levels. At each soil block, we first counted the number of morphotypes, then counted the number of root tips for each morphotype. At the single-root level, we theoretically quantified the foraging space (mycorrhizosphere volume) based on mantle length (L_{mantle}) and maximum hyphal or rhizomorphic length (L_{max}) for each morphotype. We assumed the hyphal geometric space of each EcM root tip was modeled as a cylinder. We then scaled the ectomycorrhizosphere (total hyphal geometric space) at the soil block level (10^3 cm^3) according to the number of root tips, and the weighted average space of hyphae for each soil sample at each elevational site.

$$V_{hyphae}(\mathrm{cm^3\ m^{-3}}) = \sum_{i=1}^{N} \left(\pi\left(L_{hyphae\_i} + \frac{D_{mantle\_i}}{2}\right)^2 \times L_{mantle\_i}\right) \quad \text{[Eq. 2]}$$

$$A_{hyphae}\ (\mathrm{cm^2\ m^{-3}}) = \sum_{i=1}^{N} \pi\left(L_{hyphae\_i} + \frac{D_{mantle\_i}}{2}\right)^2 \times 10 \quad \text{[Eq. 3]}$$

$$L_{hyphae}(\mathrm{cm\ m^{-3}}) = \sum_{i=1}^{N} L_{hyphae\_i} \times 100 \quad \text{[Eq. 4]}$$

Where V_{hyphae}, theoretical hyphae or rhizomorphic hyphae volume (space) per cubic soil, D_{mantle_i} is the average diameter of root covered EcM mantle for specific morphotype i, L_{hyphae_i} represents the average value of the maximum length of EcM short-hyphae or rhizomorphic hyphae for morphotype i, L_{mantle_i} represents the average length of EcM mantle belonging to morphotype i, respectively. L_{hyphae}, short-hyphae or rhizomorphic hyphae length density per cubic soil, A_{hyphae}, short-hyphae or rhizomorphic hyphae area density per cubic soil. N represents the number of morphotypes in each soil core.

Using '1/2 the diameter of the EcM root with mantle + the length of the mycelium' as the radius, we estimated the foraging area of EcM morphotype at the root tips, according to the number of root tips for each morphotype in the soil block [Eq. 3]. Hyphal length density was calculated by the hyphal length, the number of each morphotype, correspond weighting of the number of root tips in each soil block [Eq. 4].

We also evaluated the EcM extending hyphae biomass (B_{hyphae}) for each morphotype by its diameter (d_{hyphae}, mm) and maximum extending length (l_{hyphae}, mm). Following Saito (1955) and Hunt (1985), EcM mycelium biomass in the soil block was estimated using the following equation:

$$B_{hyphae}(\mathrm{g\ m^{-3}}) = \sum_{i=1}^{N} \left[\left(\pi\left(\frac{1}{2} d_{hyphae\_i}\right)^2 \times L_{hyphae\_i}\right) \times \rho \times 0.15\right] \quad \text{[Eq. 5]}$$

Where d_{mantle_i}, and L_{hyphae_i} are as defined above, ρ is the fresh weight hyphal density =1.1 g cm^{-3} (Saito, 1955), and the dry weight of mycelium is the 15% of its wet weight (Cochrane, 1958; De-Boois, 1976).

Based on the distal root tip and hyphal morphological trait measurements for each morphotype, we scaled the *Length*, *Area*, *Volume*, and *Biomass* of three hyphal exploration types in the soil block, based on a weighted count of the number of root tips associated with each EcM exploration type in the soil block (10^3 cm^3).

EcM species compositions determination.

To explore the taxonomic composition of the 51 identified morphotypes, we extracted DNA from the root tips of each EcM morphotype, correspondingly. To investigate how fungal species composition varies across different environmental conditions, we used the remaining 40 EcM root samples after morphological observation. These samples were processed for fungal DNA extraction, PCR amplification, and Illumina sequencing to identify the fungal species present in the roots.

Total genomic DNA samples were extracted using the OMEGA Soil DNA Kit (M5635-02 Omega Bio-Tek, Norcross, GA, USA), following the manufacturer's instructions, and stored at -20 °C prior to further analysis. The quantity and quality of the extracted DNA were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the fungal ITS1 region was performed using the forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the reverse primer ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components included 5 µl of buffer (5×), 0.25 µl of Fast Pfu DNA Polymerase (5 U/µl), 2 µl (2.5 mM) of dNTPs, 1 µl (10 µM) of each forward and reverse primer, 1 µl of DNA template, and 14.75 µl of ddH_2O. Thermal cycling consisted of initial denaturation at 98 °C for 5 minutes, followed by 28 cycles of denaturation at 98 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds, with a final extension at 72 °C for 5 minutes. PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After individual quantification, amplicons were pooled in equal amounts, and paired-end 2×250 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Microbiome bioinformatics was performed with QIIME2 2019.4 (Bolyen *et al.*, 2019) with slight modifications according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). Briefly, raw sequence data were demultiplexed using the demux plugin, followed by primer trimming with the cutadapt plugin (v2.3)(Martin *et al.*, 2001). Sequences were then merged, quality-filtered, and dereplicated using the functions fastq_mergepairs, fastq_filter, and derep_fullength in the Vsearch plugin. Raw sequences were quality-filtered with the following criteria: (i) the maximum mismatch ratio allowed in the overlap region of the splicing sequence was 0.1; reads that could not be assembled were discarded, and (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. All unique sequences were then clustered at 98% similarity (using cluster_size), followed by chimera removal (using uchime_denovo). Finally, the non-chimera sequences were re-clustered at 97% to generate OTU representative sequences and an OTU table. All samples were rarefied to the same number of sequences for downstream analyses based on 95% of the lowest sequence number using the "qiime feature-table rarefy" function in QIIME2 to ensure uniform sequencing depth across samples. The data were rarefied to 43,883 sequences per sample for the identification of 51 EcM morphotypes and 56,941 sequences per sample for root samples across the five elevations. Rarefaction curves indicated that our sequencing effort was sufficient when rarefied to the lowest sequencing depth (Fig. S2). We ultimately obtained 820 OTUs with 2,238,033 sequence reads for 51

EcM morphotypes and 2,468 OTUs with 2,229,733 sequence reads across the five elevational sites.

According to the OTU tables, EcM fungi species were first extracted via FUNGuild(Nguyen *et al.*, 2016). Our criteria for assigning EcM species or genus names to each morphotype included: (1) Only EcM OTUs were considered (excluding root-associated fungi such as saprotrophs, root endophytes, molds, or pathogens), with a “confidence ranking” of “highly probable,” and (2) EcM fungi where pairwise identity (i.e., the amount of nucleotide matching exactly between two different sequences) corresponding to the indicated EcM species was higher than 97%, were considered “possible” or “probable”; these EcM fungi were blasted against the UNITE database. For the 51 EcM morphotypes, we identified a total of 26 genera from 47 EcM OTUs with 164,880 sequence reads. We identified 544 EcM fungal OTUs with a total of 844,972 sequence reads among the root samples at five elevations. We used the total-sum scaling method to calculate relative abundance tables for each taxon (OTU and genus level) based on read counts across the samples. We also calculated the relative abundance of root ECM fungi by taking the ratio of sequencing reads for ECM fungi at the genus level to the total fungal sequencing reads:

$$Relative\ abundance\ (\%) = \frac{Read\ counts\ \text{of EcM fungi at genus level}}{\text{Total read counts of fungi at genus level}} \times 100\% \text{ [Eq.6]}$$

Additionally, morphotype characteristics of EcM genera were compared to reference photos from the DEEMY database (www.deemy.de/). We also referred to Agerer (2001), Lilleskov *et al.* (2011), Fernandez *et al.* (2017), and Defrenne *et al.* (2019) for the identification of soil exploration types; more details are available in Table S3. We named the soil exploration types for each EcM genus consistently with the above, i.e., contact, short-, and long-distance exploration types.

To compare EcM species diversity and morphological diversity, we uniformly calculated Simpson‘s index of diversity(Lande, 1996; Matsuda & Hijii, 2004) for both based on morphologies and OTUs at each elevational site as follows:

$$\text{Simpson‘s index of diversity} = 1 - \frac{\sum n_i(n_i-1)}{N(N-1)} \qquad \text{[Eq. 7]}$$

For morphological diversity, n_i is the root tip number of morphotype *i* in each soil sample, and N is the total number of EcM root tips in each sample. For EcM species diversity, n_i is the number of OTUs for EcM genus *i* in each sample, and N is the total number of EcM OTUs in each sample.

Absorptive fine root morphological traits measurements.

Root morphological traits were analyzed for the first two order root segments, with root diameter (RD, mm), root length, and root volume extracted from scanned images using WinRHIZO 2009. Specific root length (SRL, m g^{-1}) was calculated as root length divided by root dry mass, and root tissue density (RTD, g cm^{-3}) was calculated as the ratio of root dry mass to root volume.

Temperature data

Mean annual temperature (MAT) data for our study sites were obtained from a national gridded dataset based on observations from over 2,400 meteorological stations, developed using the "anomaly approach" (New *et al.*, 2000; Xu *et al.*, 2009). To account for elevation effects, we applied a topographic correction with a standard lapse rate of 0.65 °C per 100 m to estimate the air temperature gradient across each study area (Zhao *et al.*, 2008).

Soil properties and extracellular enzyme activity.

The air-dried soil samples were analyzed for soil organic carbon (SOC) and total soil nitrogen (SoilN) contents, and fresh soil samples were used to measure soil inorganic nitrogen (SIN) content and soil protease ($\text{Enzyme}_{\text{protease}}$) and peroxidase activities ($\text{Enzyme}_{\text{peroxidase}}$). SOC was measured using the $K_2Cr_2O_7$-H_2SO_4 calefaction method. SoilN was analyzed using the Kjeldahl digestion procedure(Gallaher *et al.*, 1976). The soil carbon to nitrogen ratio (SoilCN) was calculated from SOC and TN. Soil inorganic nitrogen (SIN), composed of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N), was extracted with KCl (1 mol L^{-1}) and measured using a continuous flow analyzer (SEAL AA3, Norderstedt, Germany). The activity of soil protease ($\text{Enzyme}_{\text{protease}}$, μmol g^{-1} soil h^{-1}) was measured using the method described by Ladd and Butler (1972), while the activity of peroxidase ($\text{Enzyme}_{\text{peroxidase}}$, μmol g^{-1} soil h^{-1}) was measured following the procedure described by Baldrian (2009) and Parham and Deng (2000).

Soil N mineralisation rate.

Soil nitrogen mineralisation was assessed using *in situ* incubation experiments conducted at five elevations, measuring the soil net nitrogen mineralization rate (N_{min}). These experiments lasted 28 days using an ion-exchange membrane extraction method (Kolberg *et al.*, 1999; Duran *et al.*, 2013). N_{min} at each elevation was calculated from the difference in soil NH_4^+-N and NO_3^--N contents before and after incubation, as follows:

$$N_{min} = \frac{(\mathrm{NO_3^-{-}N + NH_4^+{-}N})_{\mathrm{final}} - (\mathrm{NO_3^-{-}N + NH_4^+{-}N})_{\mathrm{initial}}}{t} \quad \text{[Eq. 8]}$$

Where t is the incubation time, the N_{min} was calculated as ug N g^{-1} soil d^{-1}.

Plant root ^{15}N uptake rate.

We measured three nitrogen uptake rates along the root-EcM continuum: glycine for estimating organic nitrogen, and $(NH_4)_2SO_4$ and KNO_3 for estimating inorganic nitrogen forms using ^{15}N labeling experiments. Eight trees were selected from each elevational sampling center point. The three nitrogen solutions, including glycine, $(NH_4)_2SO_4$, and KNO_3, were mixed at equal concentrations (40 μmol L^{-1} nitrogen for each form). 10 mg L^{-1} of ampicillin was added to inhibit microbial activity and prevent amino acid decomposition. 0.2 mmol $CaCl_2$ was added to maintain root metabolism during measurement (Warren & Adams, 2007; Liu *et al.*, 2017). For each tree, three labeling solutions containing one of the three ^{15}N forms (99 atom% ^{15}N-glycine, 10 atom% ^{15}N-KNO_3, or 10 atom% $(^{15}NH_4)_2SO_4$), along with one control solution containing unlabeled nitrogen forms, were applied (Liu *et al.*, 2017). The four nitrogen solutions were: ^{15}N-glycine + KNO_3 + $(NH_4)_2SO_4$, ^{15}N-KNO_3 + glycine

+ $(NH_4)_2SO_4$, $(^{15}NH_4)_2SO_4$ + glycine + KNO_3, glycine + KNO_3 + $(NH_4)_2SO_4$.

During the nitrogen labeling experiment, four absorptive root segments, each 15 cm in length, were selected from four directions around each target tree. The selected root samples were cleaned with deionized water and then immersed in four 25 ml centrifuge tubes, each containing a different nitrogen solution. After 2 hours, root samples in nitrogen solutions were excised and washed with a 50 mM KCl solution for 3 minutes, followed by a rinse with deionized water. Both labeled (N=120) and unlabeled (N=40) roots were dried at 65 °C for 48 hours until a constant weight was reached, then ground into a fine powder for $^{15}N/^{14}N$ ratio measurements using continuous-flow isotope ratio mass spectrometry (IRMS). Net nitrogen uptake rates (NUR) for each nitrogen form were calculated from data on the atom percentage of ^{15}N excess ($^{15}N_{excess}$) as follows:

$$\mathrm{NUR}\ (\mu\mathrm{g\ N\ g^{-1} root\ d.w.\ h^{-1}}) = \frac{\mathrm{N\ content} \times \frac{^{15}N_{excess}}{100}}{\mathrm{time\ (h)} \times \frac{^{15}\mathrm{N}_{tracer}}{100}} \qquad \text{[Eq. 9]}$$

Where $^{15}N_{excess}$ was the difference in atom% ^{15}N between labeled and unlabeled treatments. NUR ($\mu g\ N\ g^{-1}$ root d.w. h^{-1}) was calculated by multiplying root nitrogen content ($\mu g\ N\ g^{-1}$) by the corresponding $^{15}N_{excess}/100$, and then dividing by labeling time in hours and atom% ^{15}N of the applied ^{15}N-labeled nitrogen form ($^{15}N_{tracer}/100$) (10% for NO_3^- and NH_4^+, 98% for glycine). Organic N (NUR_{org}) and inorganic N (NUR_{inorg}) uptake rates were also calculated.

Statistical analysis.

A Sankey plot illustrated the linkages between EcM morphotypes and their corresponding fungal species. Furthermore, non-metric multidimensional scaling (NMDS) analysis was conducted using the R vegan package to compare convergence across variables of EcM root tips (including morphological diversity and the mycorrhizal colonization ratio of the three exploration types) and OTUs (including OTUs diversity and the relative abundance of the three exploration types) simultaneously. The permutational analysis of multivariate dispersions (PERMDISP) was used to examine the significance of differences between the variables of EcM morphology and OTUs.

Variance partitioning analysis was performed to quantify the importance of each factor on EcM morphological (Simpson‘s index of morphotypes diversity, mycorrhizal colonization ratio) and fungal community taxonomic characteristics (Simpson`s index of OTUs diversity, OTUs abundance) (Borcard *et al.*, 1992). We evaluated the impact of four groups: soil properties (SoilCN, N_{min}, SIN, $Enzyme_{protease,}$ and $Enzyme_{peroxidase}$); MAT; NUR_{org}, NUR_{inorg}; RD, RTD, and SRL on EcM morphological and taxonomic traits. Forward-selection model with redundancy analysis and the Akaike criterion ($p < 0.05$), using the vegan package (ordistep function) (Tudor *et al.*, 2024), identified the most relevant variables within each group.

The importance of nitrogen foraging-related variables on EcM morphology and fungal community composition was further analyzed using the “randomForest” package (Jiao et al., 2018). The importance of variables in the Random Forest (RF) model is expressed as the percentage increase in the mean squared error (%IncMSE). This value represents the increase in the prediction error of the RF model after removing the corresponding variable; the larger the value indicates the greater the importance of the predictor variable. Partial dependence plots derived from RF models were used to visualize the relationship between the response variable and each predictor variable (Wang *et al.*, 2025).

We used standardized major axis regression (SMA) to examine differences in volume density between roots and EcM hyphae in response to elevation changes. The Wald test ($p < 0.05$) was applied to assess differences in slopes between roots and EcM, as well as between short- and long-distance exploration types. A one-way analysis of variance (ANOVA) was performed to evaluate the significance of root-EcM foraging traits (e.g., length and biomass density) across elevation gradients.

We compared the variation in occurrence frequency (colonization ratio and OTU abundance) of short- and long-distance exploration types across elevations using linear regression. Additionally, we characterized the trait space by performing a principal component analysis (PCA) based on the functional traits of the short- and long-distance exploration types. The main axes of EcM functional trait variation (the first two principal components, PCs) were identified using PCA on the scaled functional traits of these hyphal exploration types, implemented with the 'prcomp' function in R. Furthermore, we identified temperature thresholds for changes in the foraging traits of roots and EcM hyphae across elevations. Indicator traits along the elevation gradient were detected, and their temperature threshold values were calculated using the R package 'threshold indicator taxa analysis' (TITAN2) (Baker & King, 2010). In this analysis, the sums of traits showing positive (z+) or negative (z-) responses to MAT were identified, and their respective threshold values were calculated.

To analyze the relationships between EcM morphological diversity (and morphotype-based colonization ratio) and EcM OTU species diversity (and OTU-based abundances) with hyphal length and volume, we examined pairwise linear relationships to compare the predictive power of morphotype-based classification versus OTU-based species information on hyphal functional traits.
To evaluate the influence of environmental factors on EcM fungal composition, we performed redundancy analysis (RDA) on the top 10 genera in terms of relative abundance. We calculated the relative distance plasticity index (RDPI) to evaluate the environmental plasticity of the root-EcM continuum (Valladares *et al.*, 2006). R (version 4.2.1) was used for all statistical analyses.

Results

The linkages between ectomycorrhizal species and morphology

We elucidated the fungal species basis for 51 morphotypes of EcM across a range of elevation gradients (Fig. 1, 2). The 51 morphotypes were associated with 820 fungal OTUs, which were assigned to 26 genera based on DNA sequencing (Table S1). It appears that many EcM genera formed one morphotype at the root tip (Fig.1), and many hyphal (few-hyphae, short-hyphae, long-rhizomorph) exploration types belong to the same genus, such as *Russula* or *Piloderma*, which can occur in any of the three types (Figs. 1, 2). Permutational analysis of multivariate dispersions (PERMDISP) revealed a significant difference between EcM morphotypes and genus-level OTUs ($P < 0.01$) (Fig.3a). These results indicate an inconsistency between the assembly of ECM morphotypes and that of species-level communities. Thus, we clarified “many-to-many" linkages between EcM morphology and species (OTUs) (Figs. 1, 2).

Furthermore, the drivers of morphology differed from those of EcM species composition (Fig. 3b, c). EcM morphological diversity was mainly explained by N-related traits (root organic N uptake rate (NUR_{org}), soil N mineralisation rate (N_{min}), and soil C: N ratio) (Fig. 3b), especially the mycorrhizal colonization of long-rhizomorph, negatively related to soil inorganic N (SIN) (Fig. S3f, S4f). In contrast, EcM species diversity was mainly related to temperature (MAT) (Fig. 3b), soil peroxidase activity ($Enzyme_{peroxidase}$), and N_{min} (Fig. 3c). Partial dependence plots predict that abundance of short-hyphae is negatively related to soil nitrogen mineralisation rate (N_{min}) (Fig. S3h, S4l), while higher abundance of long-rhizomorphic fungal taxa is positively associated with N_{min} and soil C: N ratio (Fig. S3i, S3n, o).

The quantification of ectomycorrhizal foraging space

We developed a novel method to quantify hyphal foraging space (Fig. 4a). Specifically, we measured hyphal length for each morphotype around the root tip and collected root tips from 32 soil blocks (on a unit volume basis) to scale hyphal measurements to a per cubic meter soil profile. We found that extraradical mycelium extended up to 17.5 cm from the root surface, with hyphal diameter ranging from 9 to 1200 μm (Table S2), and hyphal length ranged from 8.2×10^3 to 1.3×10^6 cm m^{-3} of soil. The theoretical maximum 3D ectomycorrhizosphere was 1.4×10^5 cm^3 m^{-3}, resulting in a 104-fold increase in soil volume exploration compared to absorptive fine roots (Table S4). Mycelial biomass was found to reach 3.5 kg m^{-3}, which was 2.3 times greater than the maximum biomass of absorptive fine roots (Fig. S5b, d; Table S4).

We then investigated variations in foraging space between hyphae and roots in response to elevation as a proxy for elevation-associated temperature change (Fig. 4a). Overall, the foraging space of both roots and EcM hyphae decreased with increasing elevation (Fig. 4b), and, the foraging space of hyphae decreased more quickly with elevation than that of absorptive fine roots (SMA slope test, $p < 0.05$; Fig. 4b). Furthermore, short-hyphae responded to elevation allometrically at a higher rate than long-rhizomorph (Fig. 4c), suggesting that they are highly adaptable and flexible to environmental change, e.g., temperature (Fig. 6c). We also found that the length of short-hyphae was positively associated with soil protease activity ($p < 0.01$), unlike long-distance mycorrhizae (Fig. 4d).

Shifts in ectomycorrhizal explorations from long to short strategies

The responses of short-hyphae and long-rhizomorph EcM hyphal exploration types to elevation differed significantly (Fig. 5): both the colonization rate and abundance of the short-hyphae type ($Abundance_{short}$) decreased with increasing elevation (Fig. 5a, b) based on both observational and sequencing data (Fig. 6a), while the abundance of EcM taxa with long-rhizomorph increased (Figs. 5b, 6a), although 51 morphotypes did not show consistent trends (Fig. 6b). Additionally, EcM morphological diversity (>0.75) was generally higher than the EcM taxonomic diversity (<0.6) and declined with increasing elevation (Fig. 5c).

There was a contrasting response of short-hyphae and long-rhizomorph to temperature change with elevation (Fig. 5b): the $Abundance_{long}$ decreased significantly when MAT reached ~3 °C, while $\text{Abundance}_{\text{short}}$ increased significantly at ~2 °C, suggesting that the short-hyphae were more sensitive to temperature change than the long-rhizomorph (Fig. 6c). The functional traits (length, volume, abundance, and MC) of EcM taxa with short-hyphae were more sensitive to temperature changes than those of the long-rhizomorph (Fig. 6c, d).

The pattern of EcM in response to elevation differed significantly for different measurements (Fig. 5a, b; Fig. 6a, b). EcM morphological traits (e.g., mycorrhizal colonization ratio) exhibited a narrow range of variation, while EcM species were relatively divergent across the elevational gradient (Fig. 3a; Fig. S3a-c).

The linkages from ectomycorrhizal species to space

Overall, the relationship between fungal OTUs and their foraging space was relatively weak (Fig. 7b, Fig. S9). Fungal species OTUs explained only 26% of the variation in foraging length for short-hyphae, while there was no significant correlation between foraging length and OTUs abundance of the long-rhizomorph (Fig. 7d). The EcM colonization ratio of the long-rhizomorph on roots was around 20-30% across five sites, while the OTUs associated with this type exceeded 60% (Fig. S3c), suggesting that the final colonization ratio may not be fully determined by their OTUs.

Discussion

We proposed the multi-dimensional ‘species-morphotype-space’ (SMS) framework. Then we found that short-hyphae become more prevalent with increasing temperature, and likely play an increasingly important role in nutrient acquisition and ecosystem C cycling, which may also lead to a decrease in long-rhizomorph EcM mycelium (Figs. 4-5). These findings suggest that climate-driven changes in EcM exploration strategies profoundly impact the biogeochemical cycles of ecosystems.

Roots feature short-hyphae as the temperature increases.

By using natural gradients of temperature based on elevation, we were able to test under natural settings how EcM morphology shifts with changes in temperature and hence predict responses to future climate warming. We found that short-hyphae increased with decreasing elevation, based on both OTUs abundance and mycorrhizal colonization, which was attributed to rising temperatures associated with decreasing elevation (Fig. 6) and increased soil inorganic nitrogen availability (Fig. 3c; Fig. S3).

These predictions from our analysis of natural gradients of temperature (Figs. 5-6) are broadly consistent with the findings of controlled experiments showing that warming favors EcM fungi with short-hyphae (Fernandez *et al.*, 2017; Steidinger *et al.*, 2019; Fernandez *et al.*, 2023). However, our results contrast with observations from latitudinal studies conducted in warmer regions (Defrenne *et al.*, 2019: 3.1–7.3°C; Jarvis *et al.*, 2013: 4.2–7.6°C). One reason is that within the colder temperature range of our study (0.24–5.2°C), modest warming provides insufficient surplus carbon to construct long-rhizomorphs. Instead, host plants prioritize allocation to short hyphae to maximize cost-benefit efficiency under both nutrient and energy limitation during the short growing season. EcM short-hyphae are sensitive and rapidly proliferate in response to rising temperatures, which aids plants in acquiring available nitrogen in warmer soils (Fig. 5d; Fig. 6). Hence, an increase in EcM short-hyphae with warming will potentially be a key mechanism for enhanced plant nutrient acquisition, soil carbon flux, and plant C storage as temperatures rise.

EcM taxa with short-hyphae can be analogies to other biological modules (Suz *et al.*, 2014), and possess unique features in their design. In particular, short-hyphae types show remarkable flexibility in construction cost and greater sensitivity to temperature changes (Fig. 6), and their behaviors can be predicted by metabolic theory (Brown *et al.*, 2004; Kwatcho Kengdo *et al.*, 2022) and the plant economics spectrum (Reich & Cornelissen, 2014; Bergmann *et al.*, 2020). Short hyphae tend to have lower construction costs (Fig. S5; Table S4), higher turnover rates (Table S4)(Ekblad *et al.*, 2013; Clemmensen *et al.*, 2015), and quick acquisitive behavior (Freschet *et al.*, 2021). They proliferate rapidly when encountering nutrient-rich patches (Rosling *et al.*, 2004; Kluting *et al.*, 2019), whereas long-rhizomorph are less responsive to transient nutrient-rich patches (Cairney, 2005; Hobbie & Agerer, 2010). Consequently, compared to long-rhizomorph, short-hyphae taxa invest less carbon in structural maintenance, yet they explore a larger soil volume than few-hyphae types (Hobbie & Agerer, 2010; Weigt *et al.*, 2012), thereby maximizing the cost-benefit efficiency in fluctuating carbon and nutrients supplies. Also, the short-hyphae not only forage but also acquire nitrogen by secreting hydrolytic proteases to "mine" and "activate" soil nutrients (Tedersoo *et al.*, 2012; Rineau *et al.*, 2016) (Fig. 4d), thereby enabling host plants to effectively adjust their nutrient acquisition strategies according to nutrient availability. Short-hyphae are also primarily hydrophilic and can efficiently capture resources from soil solutions (Hobbie & Agerer, 2010; Bahr *et al.*, 2015). In contrast, the long-rhizomorph must be hydrophobic to maintain transport channels that serve as highways for nutrients (Cairney, 2005; Hobbie & Agerer, 2010), thereby occupying nutrient patches for stable, long-term benefits.

The role of EcM morphological traits in future biogeochemical cycles

Accurate estimates of soil carbon sequestration and nutrient cycling require consideration of the relative roles and shift of long-rhizomorph hyphae in carbon storage and short-hyphae in resource uptake, as well as their responses to future climate change. Roots and associated fungi contribute 50-70% of soil carbon sequestration in EcM-dominant boreal forests (Clemmensen *et al.*, 2015). We estimated that total EcM hyphal length could be upwards of 1.3×10^{6} cm m^{-3} soil (Table S4), which is lower than the global estimate of hyphal length density in soil (1.75×10^{11} cm m^{-3}), which includes hyphae from non-mycorrhizal fungi (See *et al.*, 2022). However, the maximum mycelial biomass was more than twice that of absorptive fine roots, with 95% being attributed to long-distance hyphal types (Fig. S5, Table S4). The long-rhizomorph hyphae form hydrophobic ‘nutrient channels’ that become hotspots after decomposition (Lilleskov *et al.*, 2011; Hobbie *et al.*, 2022). These hyphae also turnover more rapidly than roots and act as a significant carbon flux, contributing to the soil carbon pool via necromass production (Ekblad *et al.*, 2013; Allen & Kitajima, 2014; See *et al.*, 2022).

Furthermore, our findings of changes in different hyphal types along the elevational gradient, point to a new mechanism explaining why soil carbon decreases with climate change (Terrer *et al.*, 2021) and nitrogen deposition (Liang & Balser, 2012) in EcM-dominated ecosystems. As temperatures rise, the increased availability of soil nitrogen—especially inorganic nitrogen in cold forest biomes (Rennenberg *et al.*, 2009; Hou *et al.*, 2023)—promotes short mycorrhizas while suppressing long mycorrhizas (Figs. 5b; 6c). This shift likely reduces soil carbon stability and pools because less mycorrhizal biomass contributes to the long-term soil carbon pool

(Ekblad *et al.*, 2013; See *et al.*, 2022; Hawkins *et al.*, 2023). However, future studies are needed to clarify the distinct ecological roles of short-hyphae and long-rhizomorph in regulating processes of soil carbon cycling.

Our findings also go beyond the traditional notion that contact-, short- and long-distance exploration types are classified by only morphological dimensions (Agerer, 2001). Our new classification and quantitative data reveal that short-hyphae demonstrate remarkable flexibility in construction (Fig. 4a), adaptability in secreting hydrolytic enzymes (Fig. 4c), and efficiency in various resource supplies (Zavišić *et al.*, 2016; Suz *et al.*, 2021), fundamentally differing from the long-rhizomorph (Suz *et al.*, 2014). Additionally, our analysis scaled the organization of extraradical hyphae from root tips to soil blocks, allowing us to quantify hyphal strategies and their potential influences on soil and ecosystem functions. Future studies on EcM nutrient exploration should take into account the diversity of morphologies at the root-tip scale, and include quantitative measurements of hyphal length, diameter, and volume.

Climate-induced EcM shifts between EcM species (OTUs) and morphology

Our findings suggest that variation in EcM species (OTUs) may not perfectly match realistic morphology across the environmental gradients in the field (Fig. 6a, b). This likely indicates that the regulation of EcM OTUs and morphology might differ (van der Linde *et al.*, 2018). EcM morphological traits are critical for assessing organic nitrogen uptake in nitrogen-limited, low-temperature environments (Defrenne *et al.*, 2019; Pellitier & Zak, 2021). We confirmed that mycelial morphological variation is primarily triggered by nitrogen-related parameters, while fungal OTUs are sensitive to temperature associated with elevation (Figs. 3, 6, S7). Particularly, the long-rhizomorph type, is linked to plant nitrogen uptake rates (Figs. S3f, S7c). However, fungal OTUs identified through sequencing characterize living, dead, and inactivated fungi, and they may not directly be involved in the resource capture process (Emerson *et al.*, 2017).

Further, the sensitivity of fungal OTUs to temperature in long-rhizomorph contrasts with the morphological traits (i.e., length) of these types (Fig. 6). Long-rhizomorph had slower turnover rates (Ekblad *et al.*, 2013), and was less sensitive to MAT (Fig. 6c, d). A snapshot of OTUs may be insufficient to describe the whole growth and decomposition processes of long-rhizomorph (Jörgensen *et al.*, 2023). In contrast, short-hyphae formed OTUs and morphological traits show similar trends and tend to be more sensitive to temperature changes (Fig. 6c). Remarkable morphological variation, from short-hyphae to long-rhizomorph (Figs. 2, 5; Table S4), enables fungi to efficiently and flexibly hunt for nutrients in heterogeneous patches (Jörgensen *et al.*, 2023; Zhu *et al.*, 2023), maintaining stability under biotic and abiotic stress (Suz *et al.*, 2014; van der Linde *et al.*, 2018). Thus, EcM fungal adaptation along elevational temperature change is more strongly associated with shifts in morphological traits, especially long-rhizomorph traits, than with changes in OTUs composition.

Under the multi-dimensional 'species-morphotype-space' (SMS) framework

The extensive quantification of morphological traits simulated nutrient foraging space and bridged the gap between OTUs structure and their foraging functions (Fig. 2), thereby serving as a steppingstone toward a holistic understanding of multidimensional mycelial diversity. Agerer (2001) proposed a classification system for EcM soil exploration types based on extensive observations. By investigating the OTUs associated with 51 diverse morphotypes, we did not find a consistent pattern in response to temperature change (Fig. 6a, b), but complex links between fungal OTUs and morphology were detected. For example, OTU abundance exhibited a clear trend along the elevational gradient (Fig. 6a), whereas morphotype patterns were not obvious (Fig. 6b). Such a mismatch between EcM species–assigned morphotypes and the morphologies expressed (Jörgensen *et al.*, 2023) likely reduces the predictability of foraging space. These results highlight complex relationships linking OTUs, morphotype expression, and final hyphal foraging behavior (Fig. 7).

We clarified the “one-genus-to-many” and “many-genus-to-one” relationships between fungal OTUs and morphology. A single fungal species or genus can exhibit few-hyphae, short-hyphae, and long-rhizomorph exploration strategies (Fig. 1, 2), and a morphotype might contain different meta-species OTUs. As a result, the OTUs-morphology linkages display ‘many-to-many’ patterns that become complex due to environmental change and the inherently facultative saprophytic nature of these fungi (Baldrian, 2009; Gray & Kernaghan, 2020). One reason for this is that EcM fungi possess saprotrophic capabilities, such that their morphologies are not strictly controlled by the host plant, and may also be induced by soil resources (Smith *et al.*, 2017). Furthermore, some EcM species-specific behaviors may be suppressed by the presence of other EcM species or by other biotic constraints, suggesting a functional separation between EcM species identity and morphological traits in nutrient foraging (Fig. S6).

The discovered diversity of morphotype complexly expressed by meta-species populations at the root tip, suggests that relying solely on OTUs may not fully predict foraging space and its response to climate change. Fungal OTUs, especially OTUs forming long-rhizomorph mycelium, may not consistently form predictable structures (Peay *et al.*, 2011; Chen *et al.*, 2018). Given that exploration type inferred from OTUs identity and those based on actual root-tip expression yielded inconsistent predictions of hyphal foraging extent, and OTUs abundance shows even lower predictive power (Figs. S8; S9), we suggest that OTUs have a limited ability to predict function due to complex interactions and processes. We proposed that the functional expression of EcM species was influenced by host plant, environment, resources, and biotic interactions (Fig. 7e) (Djemiel *et al.*, 2022), with morphology serving as a bridge between species and foraging space (Figs. 7; S8; S9). Overall, EcM taxonomic diversity was insufficient to explain the observed functional diversity and its response to temperature change.

Conclusions

Our SMS framework overcomes the limitations of previous methods and helps to clarify fundamental behavioral differences in mycelial exploration types, and the response of EcM hyphal-driven processes to temperature change associated with elevation. We found that EcM exploration strategies tend to shift from long to short strategies as temperature increases, with likely significant consequences for biogeochemical cycles. Short-hyphae can respond quickly and flexibly to changing resource availability, becoming more abundant than long-rhizomorph exploration types, thereby reducing carbon input to stable soil carbon pools. This offers a new perspective for next-generation models to predict carbon and nutrient cycling under future climate change. Furthermore, our data suggest that future predictions of belowground eco-physiological processes should quantify EcM hyphae—this hyper-flexible, hyper-diverse, and difficult-to-measure component—beyond the use of sequencing alone.

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Author Contributions

Z.M. developed the overall conceptual framework. Z.M., R.B., and L.C. designed the research and conducted the analyses. L.C. performed the experiments and collected data. Z.M., L.C., and R.B. wrote the first draft. R.B., W.Z., and L.S. substantially contributed to the revision. All authors contributed critically to the drafts and gave final approval for publication.

Competing Interests

The authors declare no competing financial interests.

Data availability

The data of this paper is available in figshare (https://doi.org/10.6084/m9.figshare.31802947).

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Figure Legends

Fig. 1 Ectomycorrhizal morphotypes are defined by one or multiple fungal species. a-c, Photographs illustrating typical morphological characteristics of the hyphal exploration types; **d**, Stacked columns represent each morphotype, corresponding to the relative abundance of ectomycorrhizal genera, indicating that a single morphotype may be composed of several genera. The right legend categorizes ectomycorrhizal genera by hyphal types (Few-hyphae, Short-hyphae, long-rhizomorph, Others), as identified in Supplementary Table 3. Morphotypes F1 to F13 represented contact exploration types; S1 to S17 represented short-distance exploration types; L1 to L21 are associated with long-distance exploration types. Morphotypes F6, F10, S4, and S12 did not associate with ectomycorrhizal OTUs based on sequencing data; “Others” denotes species with morphological characteristics not clearly defined in the current literature.

Fig. 2 The linkages of ectomycorrhizal species and morphotypes in *Abies*. The blue bars represent genera with exploration types assigned based on the literatures; once the EcM genus is known, the exploration type can be inferred accordingly. The yellow bars represent the actual morphotypes that we observed at each root tip. We identified 51 morphotypes from 53, 262 root tips, including 13 types of the few-hyphae, 17 types of the short-hyphae, and 21 types of the rhizomorphic (right, yellow color), and these morphotypes consisted of 820 OTUs from 26 genera through molecular sequencing (left, blue color). One EcM genus formed multiple exploration types (e.g., *Russula*, *Piloderma*), while a root tip covered by one morphotype was associated with several EcM genera, suggested “many-to-many” linkages between OTUs and morphotypes. Circle size represents the extended hyphal length of each morphotype measured by microscope.

Fig. 3 The drivers of ectomycorrhizal species and morphotypes in *Abies*. a. EcM morphology exhibited convergent patterns, while species composition showed remarkable variation, possibly due to different influence factors. The permutational analysis of multivariate dispersions (PERMDISP) was performed to test the significance of the difference (Average distance to centroid: EcM morphology = 0.11, EcM OTUs = 0.25; F = 71.48; $P < 0.001$); **b.** Variance partitioning was used to analyze the effects of soil, climate (i.e, mean annual temperature, MAT), nitrogen uptake rate (NUR), and root traits on the diversity of EcM OTUs and morphotypes; **c.** The importance of soil N-related variables to EcM OTUs and morphotypes in the random forest model (%IncMSE). Morphotype diversity was mostly responsive to local soil conditions (**b**) and N-related traits (organic nitrogen uptake rate, $\mathrm{NUR}_{\mathrm{org}}$; soil nitrogen mineralization rate, $\mathrm{N}_{\mathrm{min}}$; soil carbon-to-nitrogen ratio, SoilCN) (**c**), while species diversity was related to MAT and three root morphological traits (root diameter, root tissue density, specific root length) (**b**), soil peroxidase activity ($\mathrm{Enzyme}_{\mathrm{peroxidase}}$), and $\mathrm{N}_{\mathrm{min}}$ (**c**).

Fig. 4 Foraging space between roots, short-hyphae, and long-rhizomorph exploration types in response to elevations. (a), At the single-root level, we theoretically quantified the foraging space (mycorrhizosphere volume) based on mantle length ($\mathrm{L}_{\mathrm{mantle}}$) and diameter ($\mathrm{D}_{\mathrm{mantle}}$), and maximum hyphal length ($\mathrm{L}_{\mathrm{max}}$). We then scaled the ectomycorrhizosphere (total hyphal volume) at the soil block level (10^3 cm^3) according to the number of root tips and the weighted average volume of hyphae. Our ectomycorrhizosphere quantification allowed us to distinguish changes in nutrient foraging space between roots and different hyphal exploration types in response to environmental gradients. **(b),** The total volume of EcM hyphae decreased more steeply than that of absorptive fine roots with increasing elevation (EcM hyphae slope: −0.0019 vs. absorptive fine roots slope: −0.0013; SMA slope test, $p = 0.02$, N = 40), suggesting that hyphae allow quicker and more efficient soil exploration under resource-poor conditions. (**c**)**,** Thinner EcM short-hyphae showed the steepest decline across absorptive modules. However, there was no systematic change in the thicker EcM long-rhizomorph with elevation. (**d**)**,** The length of EcM short-hyphae was significantly positively correlated with protease activity ($\text{Enzyme}_{\text{protease}}$), suggesting that EcM taxa with short-hyphae play a key role in resource absorption and nutrient mobilization. *, $p \leq 0.05$; **, $p < 0.01$; *NS*, not significant. Data plotted on the x-axis and $\log_{10}$ transformed on the y-axis.

Fig. 5 The shifts for EcM short-hyphae and long-rhizomorph exploration types along elevational gradients. The colonization ratio (**a**) and species OTUs abundance (**b**) of EcM taxa with short-hyphae (Short-hyphae) decreased as elevation increased, while OTUs abundance of EcM taxa with long-rhizomorph (Long-rhizomorph) increased with elevation (**b**). Morphological diversity slightly declined as elevation increased, while taxonomic diversity showed no trends (**c**). EcM functional traits of different hyphal exploration types along the elevational gradient were analyzed by PCA ($N = 40$) (**d**). Traits associated with short-hyphae types were concentrated at lower elevations, whereas the relative abundance of long-rhizomorph ($\text{Abundance}_{\text{long}}$) was concentrated at higher elevations (**d**). Mycorrhizal colonization of short-hyphae (MC_{short}) was correlated with short-hyphae abundance ($\text{Abundance}_{\text{short}}$), whereas mycorrhizal colonization of long-rhizomorph (MC_{long}) showed no consistent relationship with $\text{Abundance}_{\text{long}}$. **, $p < 0.01$; ***, $p < 0.001$; *NS*, not significantly.

Fig. 6 The temperature sensitivity of ectomycorrhizal species and morphotypes in *Abies*. Ectomycorrhizal genera show a clear trend, with long-rhizomorph types increasing at higher elevations (**a**), while 51 morphotypes do not show consistent patterns (**b**). We employed threshold indicator analyses to assess key traits' sensitivity to mean annual temperature (MAT) (c-d). When the MAT reached 2.8°C, EcM taxa with the long-rhizomorph ($\text{Abundance}_{\text{long}}$) significantly decreased (**c**). The abundance ($\text{Abundance}_{\text{short}}$), mycorrhizal colonization (MC_{short}) of the short-hyphae type significantly increased as the temperature increased by ~2°C; hyphae length density (L_{short}), and total volume (V_{short}) of short-hyphae type, and absorptive fine root length density (L_{root}) showed an increase as the MAT reached around 3°C (**c**). The final output of accumulated z-scores in response to MAT (**d**). $\mathbf{z^+}$**:** Positively responding indicator groups (five root- and short-hyphae- related traits in panel **c**) increased rapidly around ~1.5 °C and peaked ~3 °C, indicating high temperature sensitivity. $\mathbf{z^-}$**:** Negatively responding indicator groups ($\text{abundance}_{\text{long}}$ in panel c) declined around ~2 °C and reached minima near 4 °C, indicating greater temperature tolerance. Filled symbols indicate $\text{Abundance}_{\text{long}}$ that declined with increasing MAT (↓), while open symbols represent five traits that increase (↑). The size of the symbols is proportional to the magnitude of the response (z-score). Horizontal lines represent the 5th and 95th quantiles of values, indicating the largest changes in taxon z-scores among 500 bootstrap replicates.

Fig. 7 The framework to understand the linkages from species, morphology to function. At the soil block level, the Simpson diversity index of morphotypes was significantly related to hyphal foraging volume (**a**), whereas OTUs showed no relationship with foraging volume (**b**). Specifically, mycorrhizal colonization (MC) of long-rhizomorph types was positively related to their hyphal length (**c**), whereas OTU abundance of short-hyphae types was positively correlated with the hyphal length of this type (**d**). We propose a framework in which fungal DNA detected by molecular sequencing undergoes different screening or interpretations (valves) to express mycelial morphological characteristics and functional space (**e**). This framework links OTUs with final morphotypes (linkage 1) and simulated morphology-foraging space relationships (linkage 2), suggesting why there are complex linkages from OTUs to functional foraging space (linkage 3). Data plotted on the x-axis and log transformed on the y-axis (**a-d**).

Extended Data Table 1 List of absorptive fine root and ectomycorrhizal functional traits, plant physiological traits, and environmental factors.

Traits		Unit	Abbreviations
Root traits	Root volume density	cm^3 m^{-3}	V_{root}
	Root length density	10^3 cm m^{-3}	L_{root}
	Root biomass	g m^{-3}	B_{root}
	Specific root length	m g^{-1}	SRL
	Root diameter	mm	RD
	Root tissue density	gcm^{-3}	RTD
EcM traits	EcM mycelium length density	10^3 cm m^{-3}	L_{EcM}
	EcM mycelium volume density	10^3 cm^3 m^{-3}	V_{EcM}
	Total volume of short-hyphae type	10^3 cm m^{-3}	V_{short}
	Total volume of long-rhizomorph type	10^3 cm m^{-3}	V_{long}
	Length density of short-hyphae type	10^3 cm m^{-3}	L_{short}
	Length density of long-rhizomorph type	10^3 cm m^{-3}	L_{long}
	Biomass of short-hyphae type	g m^{-3}	B_{short}
	Biomass of long-rhizomorph type	g m^{-3}	B_{long}
	Mycorrhizal colonization ratio of the few-hyphae type	%	$MC_{\mathrm{few\text{-}hyphae}}$
	Mycorrhizal colonization ratio of the short-hyphae type	%	MC_{short}
	Mycorrhizal colonization ratio of the long-rhizomorph type	%	MC_{long}
	Relative abundance of OTUs of few-hyphae type	%	$\mathrm{Abundance}_{\mathrm{few\text{-}hyphae}}$
	Relative abundance of OTUs of short-hyphae type	%	$\mathrm{Abundance}_{\mathrm{short}}$
	Relative abundance of OTUs of long-rhizomorph type	%	$\mathrm{Abundance}_{\mathrm{long}}$
Physiological traits	root organic N uptake rate	ug g^{-1} h^{-1}	$\mathrm{NUR}_{\mathrm{org}}$
	root inorganic N uptake rate	ug g^{-1} h^{-1}	$\mathrm{NUR}_{\mathrm{inorg}}$
Environmental factors	Mean annual temperature	°C	MAT
	Soil carbon to nitrogen ratio	—	SoilCN
	Soil N mineralization rate	ug g^{-1} d^{-1}	N_{min}
	Soil protease activity	ug g^{-1} d^{-1}	$\mathrm{Enzyme}_{\mathrm{protease}}$
	Soil peroxidase activity	ug g^{-1} d^{-1}	$\mathrm{Enzyme}_{\mathrm{peroxidase}}$
	Soil inorganic N concentrations (NH_4^+-N+NO_3^--N)	mg	SIN
	Soil water content	%	SWC

Supporting Information:

Fig. S1 Measurements of morphological traits at a single ectomycorrhizal-root system.
Fig. S2 Rarefaction curve of fungal OTUs of the 51 ectomycorrhizal morphotypes and samples across the five elevational sites.
Fig. S3 Patterns and influence factors of ectomycorrhizal colonization and OTUs abundance for three hyphal exploration types along elevational gradients.
Fig. S4 The positive and negative relationships between ECM morphotype- and OTU-related metrics and soil nitrogen-related variables.
Fig. S5 Variation of ectomycorrhizal traits and absorptive fine root traits in elevational sites.
Fig. S6 Comparison of hyphal exploration types identified between ectomycorrhizal morphology and OTUs.
Fig. S7 Influence of environmental factors on variation in ectomycorrhizal morphological traits and operational taxonomic units (OTUs).
Fig. S8 The ectomycorrhizal foraging space and biomass can be explained by EcM morphological traits.
Fig. S9 Relationships between ectomycorrhizal species (OTUs) and hyphal traits of short-hyphae and long-rhizomorph types.

Table S1 Ectomycorrhizal OTUs composition (at genus level) of 51 morphotypes.
Table S2 Morphological dimensional properties of root tips of 51 morphotypes.
Table S3 Hyphal exploration types identified by ectomycorrhizal genus found in the study sites.
Table S4 Descriptive statistics of EcM traits of Faxon fir in soil blocks across the five elevations.
Table S5 Results of Standard Major Axis regressions (SMA) test between root-ectomycorrhizal traits and elevation and linear regression analysis of the ectomycorrhizal hyphae length with soil protease activity, based on different data transformations.