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Research

Ecological and genomic variation in ectomycorrhizal fungal exploration types

Thomas M. Mansfield[1], Artin Zarsav[2,3], Filipa Cox[4], Laura M. Suz[5,6], Martin I. Bidartondo[5,7], Sietse van der Linde[8], Nadia Barsoum[9], Colin Averill[3,10], Alan Kuo[11], Leho Tedersoo[12], Pasi Rautio[13], Arthur Gessler[2,3], Bruno De Vos[14], Luc Croisé[15], Henning Meesenburg[16], Markus Wagner[16], Frank Jacob[17], Paweł Lech[18], Anna Kowalska[18], Martin Greve[19], Genoveva Popova[20], Beat Frey[2], Marcus Schaub[2], Marco Ferretti[2], Peter Waldner[2], Vicent Calatayud[21], Roberto Canullo[22], Giancarlo Papitto[23], Aleksander Marinšek[24], Lars Vesterdal[25], Morten Ingerslev[25], Helge Meissner[26], Volkmar Timmermann[26], Nadine Eickenscheidt[27], Andreas Schmitz[27,28], Francis M. Martin[29,30], Joseph Spatafora[31], Peter G. Kennedy[32], Annegret Kohler[29], Jonathan M. Plett[33], Ian C. Anderson[33], Sara Branco[34], Igor V. Grigoriev[11,35], Chris J. Pires[36], Sarah A. Unruh[37], Lawrence W. Zettler[38], Otto Miettinen[39], Ilya Viner[39], Tom W. May[40], Teresa Lebel[41,42], David E. A. Catcheside[41], Pamela S. Catcheside[41,42], Helen P. Vonow[42], Leigh A. Burgoyne[41], Julia Haska[42] and Mark A. Anthony[1]

[1]Center for Microbiology and Environmental Systems Science, University of Vienna, Vienna, 1030, Austria; [2]Swiss Federal Institute for Forest, Snow, and Landscape Research WSL, Birmensdorf, 8093, Switzerland; [3]Department of Environmental Systems Science, ETH Zürich, Zürich, 8092, Switzerland; [4]Department of Earth and Environmental Sciences, The University of Manchester, Manchester, M13 9PT, UK; [5]Royal Botanic Gardens, Kew, Richmond, TW9 3DS, UK; [6]Institut Botànic de Barcelona (IBB), CSIC-CMCNB, Passeig de Midgia s.n., Barcelona, 08038, Spain; [7]Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK; [8]Netherlands Institute for Vectors, Invasive Plants and Plant Health, Netherlands Food and Consumer Product Safety Authority, Wageningen, 6706 EA, the Netherlands; [9]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK; [10]Funga Public Benefit Corporation, Austin, TX 78702, USA; [11]U.S. Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; [12]Mycology and Microbiology Center, University of Tartu, Tartu, 50409, Estonia; [13]Natural Resources Unit, Natural Resources Institute Finland, Rovaniemi, 96200, Finland; [14]Research Institute for Nature and Forest, Brussels, 1000, Belgium; [15]French National Forest Office, Fontainebleau, 77300, France; [16]Northwest German Forest Research Institute, Göttingen, 37079, Germany; [17]Saxon State Office for Environment, Agriculture and Geology, Dresden, 01326, Germany; [18]Forest Research Institute, Sękocin Stary, 05-090, Poland; [19]Forest Monitoring, Research Institute for Forest Ecology and Forestry FAWF, Trippstadt, 67705, Germany; [20]Executive Environmental Agency at the Ministry of Environment and Water, Sofia, 1618, Bulgaria; [21]Fundación CEAM, Paterna, 46980, Spain; [22]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, 62032, Italy; [23]Arma dei Carabinieri Forestry Environmental and Agri-food Protection Units, Rome, 00187, Italy; [24]Slovenian Forestry Institute, Ljubljana, 1000, Slovenia; [25]Department of Geosciences and Natural Resource Management, University of Copenhagen, Frederiksberg C, 1958, Denmark; [26]Norwegian Institute of Bioeconomy Research NIBIO, Ås, 1431, Norway; [27]North Rhine Wesphalia Office for Nature, Environment and Climate, Recklinghausen, 45659, Germany; [28]Thünen Institute of Forest Ecosystems, Eberswalde, 16625, Germany; [29]INRAE, UMR 1136 Interactions Arbres-Microorganismes, Centre INRAE Grand Est-Nancy, Université de Lorraine, Champenoux, 54280, France; [30]The National Key Laboratory of Ecological Security and Sustainable Development in Arid Region, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, 730000, China; [31]Department of Botany and Plant Pathology, Oregon State University, Corvalis, OR 97331, USA; [32]Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN 55108, USA; [33]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia; [34]Department of Integrative Biology, University of Colorado Denver, Denver, CO 80204, USA; [35]Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA; [36]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, 80523-1170, USA; [37]College of Biological Sciences, University of Missouri, Columbia, MO 65211-7400, USA; [38]Department of Biology, Illinois College, 1101 West College Ave, Jacksonville, IL 62650, USA; [39]Finnish Museum of Natural History, University of Helsinki, Helsinki, 00100, Finland; [40]Royal Botanic Gardens of Victoria, Melbourne, Vic., 3004, Australia; [41]Biogical Sciences, College of Science and Engineering, Flinders University, Adelaide, SA, 5001, Australia; [42]State Herbarium of South Australia, Adelaide, SA, 5000, Australia

Authors for correspondence:
Thomas M. Mansfield
Email: thomas.malcolm.mansfield@univie.ac.at

Summary

• Ectomycorrhizal fungi (EMF) produce mycelia with variable extension and complexity, which can be classified according to soil 'exploration types' (ETs). ETs have received attention as one of the few mycorrhizal trait frameworks, but without an empirical classification of ET functional diversity and environmental preferences, understanding and interpreting EMF biogeographic patterns has been difficult.

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Mark A. Anthony
Email: mark.anthony@univie.ac.at

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• We conducted a synthesis combining: comparative EMF genomics to describe functional divergence in decomposition and nutrient cycling genes across ETs; and EMF trait distribution modeling across continental Europe, pairing soil and root EMF surveys to establish biogeographic ET niche profiles.
• We demonstrate a signature of ETs encoded in EMF genomes, which is independent from phylogeny and linked to biomass production strategies. EMF ET relative abundances were separated by soil, root, and dominant tree leaf type habitats and exhibited unique correlations with forest biotic (e.g. plant productivity and plant pathogen densities) and abiotic (e.g. nitrogen deposition and soil pH) conditions.
• These findings support a theory that EMF niche partitioning can be partially explained by extraradical mycelial traits, with underlying variation in ET biogeography likely arising from distinct decomposition and nutrient cycling potentials. We also identify important limitations to this trait framework and provide a guided outlook for future research.

Introduction

Ectomycorrhizal fungi (EMF) are a diverse group of tree root-associated fungal symbionts. They help trees recruit offspring (Bennett *et al.*, 2017; Liang *et al.*, 2020), take up nutrients (Read & Perez-Moreno, 2003), and displace plant pathogens (Buscot *et al.*, 1992), with effects that scale from the individual tree to entire forest functioning (Phillips *et al.*, 2013; Anthony *et al.*, 2024). A key driver of EMF activity is the growth and exploration of extraradical mycelium. Each year, EMF produce hundreds of kilograms of extraradical mycelium per forest hectare (Ekblad *et al.*, 2013), making them one of the dominant fungal guilds in forest soils (Hagenbo *et al.*, 2024) and important contributors to overall ecosystem functioning (Averill & Hawkes, 2016; Köhler *et al.*, 2018; Liang *et al.*, 2020; Lindahl *et al.*, 2021; Read & Perez-Moreno, 2003). The extraradical mycelium of EMF releases enzymes to decompose and take up otherwise inaccessible soil nutrients (Landeweert *et al.*, 2001; Hobbie & Hobbie, 2008; Hobbie & Agerer, 2010), and EMF host plants allocate *c.* 2.5 petagrams of carbon per year to EMF mycelium globally (Hawkins *et al.*, 2023). What controls variation in EMF extraradical mycelium development can therefore shape forest nutrient cycling, decomposition, and the symbiotic functioning of EMF and their host plants.

Extraradical mycelium traits influence EMF functioning in ways analogous to the networks formed by wood decomposing fungi to acquire and transport resources (Boddy *et al.*, 2009). For EMF, these traits can be classified into broad sets of extraradical mycelium characteristics known as 'exploration types' (hereafter: ETs). ETs are generally based on the morphology of hyphae and rhizomorphs emanating from colonized roots (ectomycorrhizas) and represent the structure, amount, and form of extraradical mycelium (Agerer, 2001) (see Fig. 1, for a description). Some ETs, such as many formed by species within the charismatic genera *Russula* and *Cenococcum*, produce hydrophilic mycelium adapted to rapidly absorbing mineral nutrients (Hobbie & Agerer, 2010). Other well-known genera, such as *Cortinarius* and *Suillus*, form hydrophobic mycelium capable of uniting into chord-like rhizomorphs that absorb and transport water and nutrients from resource-rich to -poor areas (Cairney, 2005; Hobbie & Agerer, 2010; Hobbie *et al.*, 2024). ETs can also be classified to reflect variation in overall extraradical mycelial biomass into 'long' or 'short' distance groups (Khalfallah *et al.*, 2024) or by hydrophobicity (Lilleskov *et al.*, 2011), which can reveal unique insights into their fine-scale distributions in the soil profile (Genney *et al.*, 2006). ETs are also correlated with important ecosystem functions, including nitrogen (N) cycling (Hobbie & Högberg, 2012), extracellular enzyme production (Tedersoo *et al.*, 2012), host photosynthetic rates (Fernandez *et al.*, 2017), and forest tree growth (Anthony *et al.*, 2022). While additional EMF traits such as successional type and N preference are also needed to characterize EMF linkages to the ecosystems they inhabit (Jörgensen *et al.*, 2025), ETs are currently the only trait specific to extraradical mycelium classification. Yet, like most ecological traits, ETs must be evaluated carefully due to significant gaps in our understanding of ET distributional patterns and functions (Jörgensen *et al.*, 2023; Trocha *et al.*, 2021, p. 202) (Box 1).

Despite theoretical development and extensive research on EMF ETs within particular forest stands and global change contexts (e.g. most notably in relation to N deposition; Lilleskov *et al.*, 2011; van der Linde *et al.*, 2018), what empirically defines broader ET ecological patterns has not kept afoot. Based on insights largely developed from analyses of individual fungal lineages, capacities for decomposition and nutrient cycling have been proposed to vary across ETs (Lilleskov *et al.*, 2019). For example, the large number of class II peroxidases within the genus *Cortinarius* (Kohler *et al.*, 2015) has led to the idea that medium-distance fringe (MD-F) EMF may be stronger decomposers than other ETs, and while there is also supporting stable isotope evidence (Hobbie & Agerer, 2010), it remains an open question for most members of this ET as well as for other high biomass producing ETs from the Basidiomycota (e.g. long-distance; LD), which are sometimes combined with MD-F into a single, albeit distinct 'long-distance' category (Khalfallah *et al.*, 2024). Furthermore, host tree species is a large-scale driver of EMF diversity (van der Linde *et al.*, 2018) and ET abundances (Rosinger *et al.*, 2018), but this is typically not accounted for when studying ET biogeography at small spatial scales. These outstanding questions demonstrate the limits of small-scale and

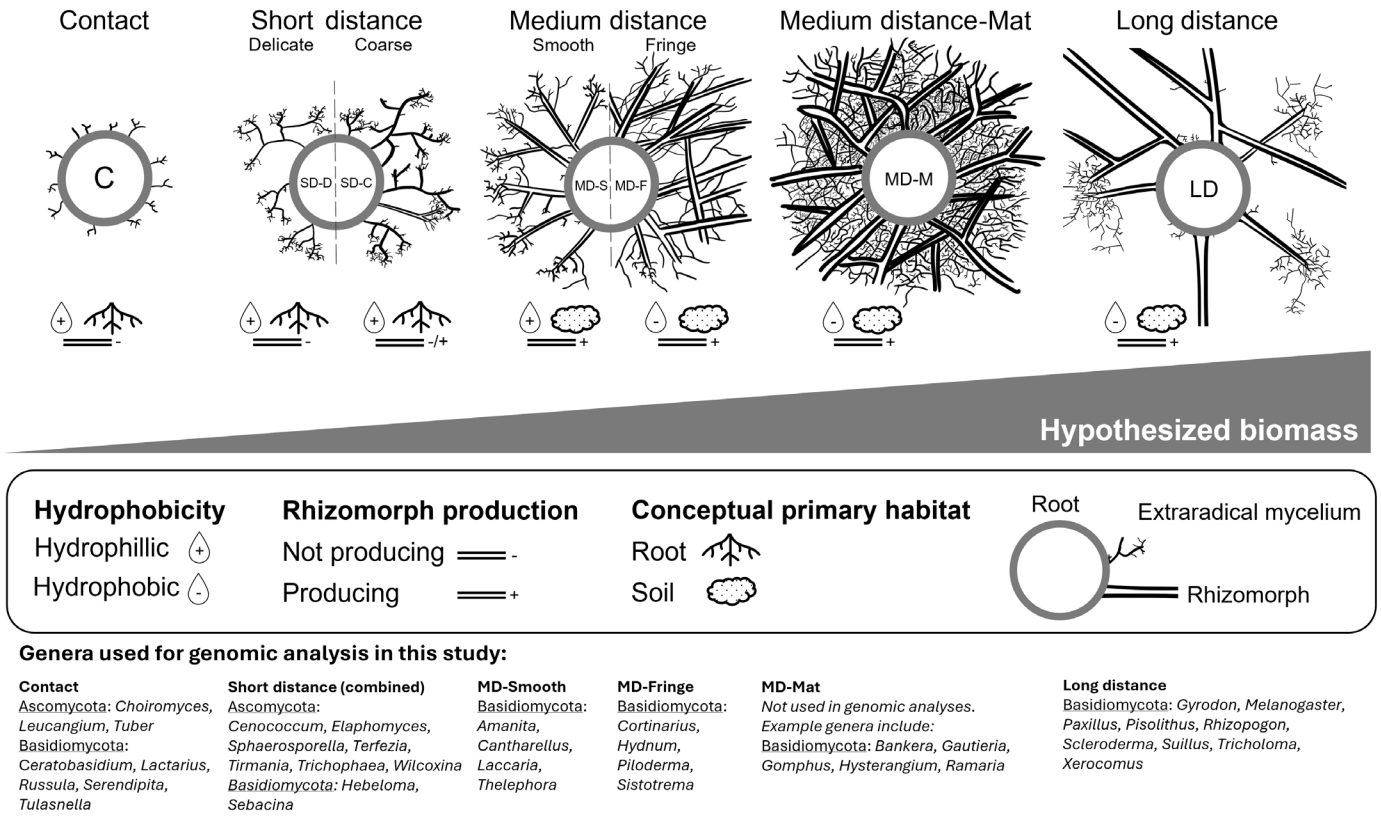

Fig. 1 Characteristics of ectomycorrhizal fungal exploration types. Note that traits are based on morphological observations of mycorrhizal root tips, and there is a degree of plasticity to at least some of these categories. Some genera utilize multiple exploration types depending on the species. Diagrams are conceptualized based on Agerer (2001, 2006) and Põlme *et al*. (2020) and are intended to be representations of general morphological features. Genera that were used for genomic analysis in the present study are listed, except for medium-distance mat, which was not used.

species-specific approaches to understanding EMF traits. To overcome this, we conducted a first-of-its-kind, large-scale genomic and ecological analysis of the differences among ET functional attributes and their linkages to environmental conditions and forest functioning.

In this study, we applied a synthesis of genomic and species distribution modeling techniques to empirically characterize the ecological variation of ETs. First, we tested how genomic variation in EMF genes involved in decomposition as well as N uptake and metabolism (hereafter: N cycling) varied across ETs using a comparative genomic approach. To our knowledge, this is the first study to test whether there is a genomic basis to ETs. We hypothesize that EMF functional genes involved in decomposition and N cycling vary significantly across ETs, and that high biomass ETs (particularly MD-F and LD) have the greatest genomic capacities for decomposition and N cycling. Next, we modeled distributions of EMF ETs at a continental scale using two complementary European datasets of root and soil dwelling EMF paired with a large and comprehensive set of *in situ* biotic and abiotic forest variables, including climate, N deposition, forest productivity, age, foliar nutrients, and soil physicochemical properties. We hypothesize that high biomass EMF ETs are more abundant in soils than on root tip, while low biomass ETs are more abundant on roots than in the surrounding soils. We also hypothesize that EMF ETs are distributed according to different sets of environmental conditions beyond N deposition (Box 1) and that these environmental predictors would be more similar for ETs harboring similar traits (e.g. rhizomorph formation; Fig. 1).

Materials and Methods

Genomic data collection and processing

Genomic data on protein families (i.e. Pfams) were derived from fully sequenced EMF fungal genomes from the Joint Genome Institute's (JGI) MycoCosm (Grigoriev *et al*., 2014). We used protein family (Pfam) models from the annotation portal, which assigns protein domains to predicted genes based on hidden Markov models. To explicitly control for variation in genome size, we calculated proportions of Pfams relative to the total (Treseder & Lennon, 2015). We first examined total Pfam profiles and then split the data frame into specific categories involved in decomposition and N cycling. Because EMF have a significant potential to influence forest N cycling dynamics (Read & Perez-Moreno, 2003; Franklin *et al*., 2014), we focused on Pfams involved in diverse N cycling pathways. We targeted our analysis of decomposition on plant biomass decay pathways and included

Box 1. An overview of the challenges identified in understanding the ecological niches of exploration types.

Challenge	Representative examples	Explanations
Inconsistencies in where some exploration types (ETs) are expected to grow (i.e. primarily on roots, surrounding soil, or both) (Agerer, 2001) and where they are observed to be most abundant, raising questions about mycelial extent and biomass production amounts. A general assumption for in-growth bags is that high biomass and rhizomorph producing ETs should be at high relative abundances while low biomass ETs should be comparatively rare. Lower biomass producing ETs, like the contact-type, are expected to be more common on roots than in soils and vice versa for higher biomass producing ETs.	(**1**) Some studies report expected patterns whereby high biomass and rhizomorph producing ETs are more abundant in soil than on roots (Genney *et al*., 2006; Kjøller, 2006; Parrent & Vilgalys, 2007). This supports the idea that ETs display contrasting extent and biomass production amounts. (**2**) Expected patterns in ET distributions are not consistently observed within in-growth bags, soil, or on roots (Jörgensen *et al*., 2023). This suggests that foraging variation among ETs does not consistently hold between local environments and/or methodological problems.	(**1**) In-growth bags are often placed in the soil immediately beside root systems, and thus even low biomass ETs do not require substantial extraradical mycelium growth for colonization. (**2**) Formation of emanating hyphae and rhizomorphs can show morphological plasticity (van der Linde *et al*., 2018; Suz *et al*., 2021) and can change during EMF ontogeny (Agerer, 2001). (**3**) Not all species within the same genera fit a single ET, and there are even finer levels of variation within rhizomorph subtypes than those classified by ET groupings (Pritsch *et al*., 1997; Agerer, 2001). (**4**) ET classifications may be unreliable, especially in less-studied forests, where well-known genera can include EMF species whose ETs have not yet been described. It is typical that ETs are indirectly assigned at the genus level in DNA (meta)barcoding studies, and while this may adequately capture mycelium development in some systems where taxa have been well-described, this is less straight-forward in understudied ecosystems.
Resource capture responses of nitrophobic and nitrophilic taxa to N enrichment do not always follow expected patterns defined by ET groupings. As a response to high nitrogen (N) availability in historically N limited systems, ectomycorrhizal fungi (EMF) that produce large amounts of biomass are expected to be at lower abundances because they are hypothesized to be a higher carbon cost to host plants who could otherwise take up N without mycorrhizas (Lilleskov *et al*., 2011; Suz *et al*., 2014; Defrenne *et al*., 2019; Khalfallah *et al*., 2024). Some of these fungi may even have a reduced capacity for inorganic N uptake compared to putatively nitrophilic taxa (Nygren *et al*., 2008).	(**1**) There are contrasting responses to both ambient and enriched N conditions within assigned ETs (Lilleskov *et al*., 2011), and *in situ* responses to N enrichment are not always as expected based on theory (Karst *et al*., 2021; Trocha *et al*., 2021). For example, 13 years of experimental N additions doubled the relative abundance of high biomass producing EMF in a Canadian boreal forest (Karst *et al*., 2021). This surprising result raises the idea that N additions are not a universal negative driver of high biomass EMF ETs. If N colimits EMF growth (alongside plant hosts), then N additions may stimulate high biomass EMF ETs (Franklin *et al*., 2014). Although, responses may also be mediated by background N deposition levels (Moore *et al*., 2021). (**2**) EMF taxa considered to be nitrophobic, including some high biomass taxa such as *Suillus*, grow better under enriched N conditions (Chen *et al*., 2024). Notably, only those capable of proteolysis grow well on more complex proteinaceous substrates (Lilleskov *et al*., 2002). This suggests that N acquisition may be unlinked to extraradical mycelium characteristics.	(**1**) Most studies to date are statistically underpowered and do not account for other predictor variables that may have interactive or even stronger effects on EMF ETs compared with N inputs, such as plant productivity, root biomass, soil pH, P inputs, soil texture, and climate (Peay *et al*., 2011; Suz *et al*., 2021; Anthony *et al*., 2022), which could obscure EMF ET responses to N additions. For example, experimental N additions alone did not select for high biomass producing ETs at the Harvard Forest, but with concurrent warming and N additions, high biomass EMF taxa became dominant (Anthony *et al*., 2021). Simultaneous consideration of relevant drivers alongside forest N indicators may reveal common trends within ET groupings that transcend taxa-specific or within-species variation in responses. (**2**) There is variation to N use among EMF taxa within an ET, suggesting more plasticity to N sensitivity within ETs than originally theorized (Almeida *et al.*, 2019). (**3**) Assignments to ETs may be incorrect for some EMF and this blurs results (see Comment 4 for elaboration).

Pfams with annotations for decomposing cellulose, xylans, xyloglucans, mannans, pectin, inulin, starch, and lignin (see Supporting Information Table S1, for a list of enzymes from Benocci *et al*., 2017). For N cycling, we included Pfams with annotations involved in lignin depolymerization even if they are also part of the overall decomposition group (i.e. oxidative enzymes), chitin degradation, peptidases, proteases, amino acid lyases, nitrite/nitrate reductases, and N transporters. ETs were assigned to EMF at the genus level using the FungalTraits database (Põlme *et al*., 2020) and DEEMY (Information System for Characterization and DEtermination of EctoMYcorrhizae, http://www.deemy.de/). EMF could be assigned one of seven ETs: contact (C), short-distance delicate (SD-D), SD coarse (SD-C), medium-distance smooth (MD-S), MD fringe (MD-F), MD

mat (MD-M), and LD (Fig. 1). In total, the analysis was conducted on 106 individual genomes with groups from the Basidiomycota (90 genomes) and Ascomycota (16 genomes). These groups were selected because they include all genomes either publicly available or made available with permission by JGI principal investigators. For this analysis, we did not split the SD group into SD-D and SD-C because there were too few observations (10 genomes) (see Fig. 2; Table S2, for a full list of genomes). We generated a whole-genome-based phylogenetic tree following the standard JGI MycoCosm pipeline. We identified sets of orthologous protein sequences across the genomes using BLASTp. Orthologs were then grouped using MCL and aligned using MAFFT to approximate a maximum likelihood phylogenetic tree using FastTree (Fig. S1).

Biogeographic data collection and processing

EMF relative abundance data was sourced from root (Cox *et al.*, 2010; Suz *et al.*, 2014; van der Linde *et al.*, 2018) and soil (Anthony *et al.*, 2024) community datasets. Both datasets are from samples collected at intensively monitored International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) Level II plots in Europe (Ferretti & Fischer, 2013). The root tip dataset is based on mycorrhizal root tips using full-length internal transcribed spacer (ITS) barcoding across 137 plots in 20 European countries. The soil community dataset is based on ITS fungal DNA metabarcoding across 238 plots in 15 countries. At 50 of the plots distributed widely across Europe (see map in Fig. S2), there are matching root and soil data; although specific sampling times and locations within plots differed, these data are comparable at a plot level. Raw, full-length ITS sequences were first downloaded and passed through bioinformatic quality control measures; the ITS region was excised; operational taxonomic units (OTUs) were clustered at a 98% sequence similarity threshold, and OTUs were assigned taxonomy against the UNITE database (https://unite.ut.ee/), with complete details for the root (Anthony *et al.*, 2022) and soil (Anthony *et al.*, 2024) analyses described elsewhere. EMF were

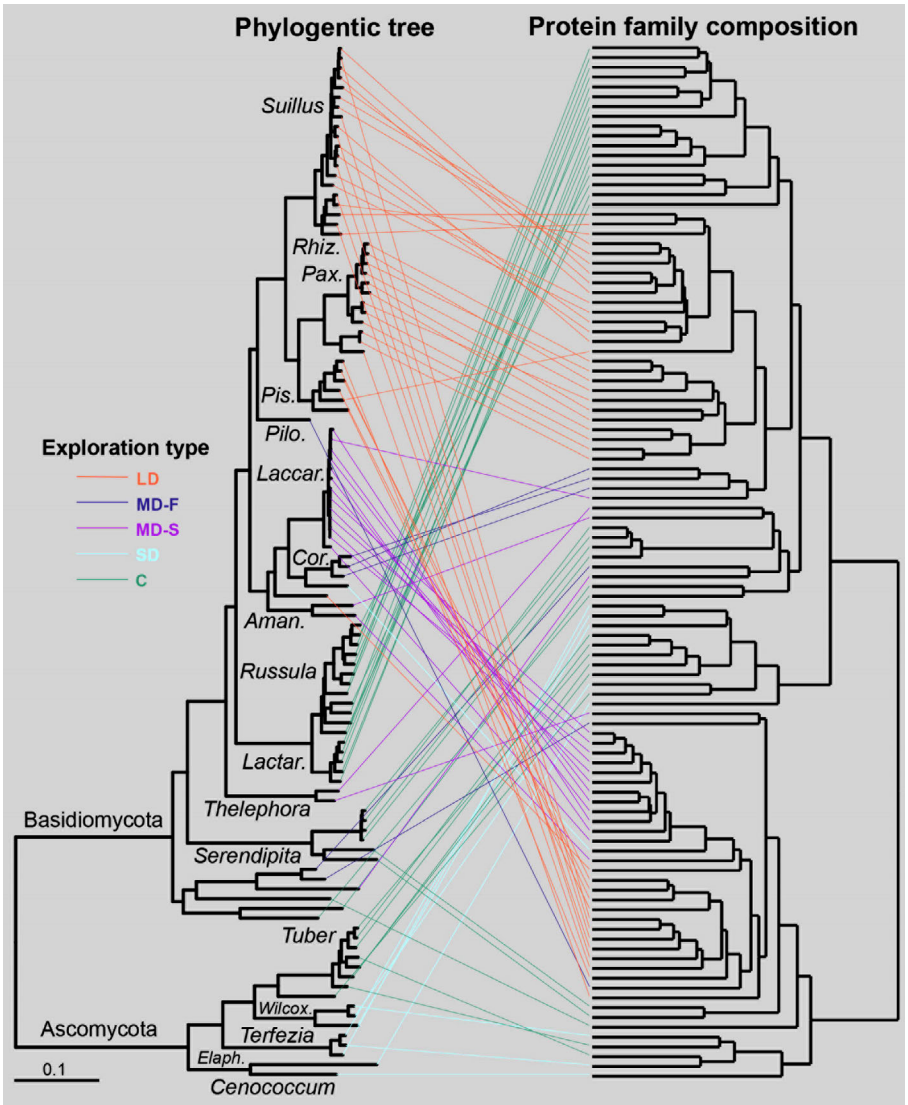

Fig. 2 Link between ectomycorrhizal fungal phylogeny and protein-family composition across exploration types. Phylogenetic tree showing the distribution of ectomycorrhizal fungi included in our genomic analysis (see the Materials and Methods section, for details on tree construction). Species names are not included due to legibility issues but select genera from each major lineage are included to facilitate inference (see Supporting Information Fig. S1, for a tree with each species name included). On the right is the protein-family composition visualized as a dendrogram. Linkages between phylogeny and protein-family composition are visualized by connecting lines.

assigned to ETs as described previously for genomic data. From both datasets, non-EMF were excluded, and the proportion of ETs was estimated from total EMF sequences. Separation of EMF from other fungal guilds is important for the soils dataset because we compare relative abundances of ETs on root tips, where there were only EMF, to soils where there are many different fungal ecological guilds (will be discussed later), and to make these comparisons equal, we controlled for the presence of non-EMF in soils when comparing EMF proportions. For the soils dataset alone, the proportions of soil pathogens and saprotrophs were also included as covariates, calculated before removing the EMF subset of sequences to account for the proportional nature of the data. Pathogenic and saprotrophic guilds were assigned using FUNGuild (Nguyen *et al.*, 2016), and we included all probable or higher level designations. Genera with > 1 functional guild assignment (e.g. plant pathogen-saprotroph) were assigned to each category. In other words, a genus in the soil dataset could be assigned to > 1 discrete functional guild. In total, 43% of the saprotrophic and 98% of the plant pathogenic OTUs were assigned to > 1 functional guild.

Metadata processing

Because sampling was conducted at ICP Forests Level II monitoring plots, we used forest inventory and soil fungi data collected from the same plots to predict EMF ET distributions. Covariables included the dominant tree leaf type (defined as the most abundant tree leaf type within the monitoring plot; Gymnosperm 'needleleaf' vs Angiosperm 'broadleaf'), forest age, and plant productivity, calculated using periodic diameter at breast height measurements and allometric equations (t C ha^{-1} yr^{-1}). Foliar N and phosphorus (P) content of sun-exposed leaves and needles from the upper third part of the tree crown of typically 5 trees of the main tree species were determined using dry combustion and inductively coupled plasma emission spectroscopy (ICP-AES), respectively (mg N or P g^{-1}). Topsoil N stocks (0–10 cm depth) were calculated using measurements of total N concentration, bulk density, and sampling depth (t N ha^{-1}). Soil pH was measured on soil slurries with deionized (DI) water (10 g soil : 20 ml DI water). Soil clay content (%) was measured based on the pipette method (0–2 μm fraction). Mean annual temperature (MAT), precipitation (MAP), and N deposition rates (average between 2010 and 2015 for the root tip data; 2019 for the soils data to align with the time of sampling) were obtained from WorldClim (https://worldclim.org/) and Emep (https://emep.int/) at a 1-km^2 spatial resolution, respectively.

Statistical analysis

All statistical analyses were conducted in R, and significance was set to $P < 0.05$. Variation in the proportion of Pfams was analyzed using distance-based redundancy analysis (db-RDA).

We performed db-RDA using the capscale function in the vegan package (Dixon, 2003) to test the effects of ET, fungal phylogeny, total number of genes in the genome, and genome size. Number of genes refers to the total number of predicted protein-encoding genes in the genome and genome size represents the total length of the assembled genome. We used the vif.cca function in the car package (Fox *et al.*, 2001) to evaluate variance inflation factors to assess independence between ET and other possible explanatory variables. To account for possible covariation between phylogeny and ETs, we included phylogeny as a 'Condition' term to perform partial db-RDA. This enabled us to partial out variation in Pfams that was explained by phylogeny before testing the effects of ETs. To conduct multiple comparisons, we generated pairwise db-RDA models and used the adjust function with method = 'bonferroni' to account for false discovery.

Phylogeny was represented in our models by calculating the pairwise evolutionary distances between taxa in our phylogenetic tree using the cophenetic function. This was converted to a Euclidean distance matrix and analyzed using principal coordinates analysis (PCoA) using pcoa function in the ape package (Paradis *et al.*, 2002). PCoA1 captured 88.6% of the variation and was therefore used as a covariable to represent fungal phylogeny. This was further validated by regressing pairwise evolutionary distance in the phylogenetic tree with pairwise differences in PCoA1 ($R^2 = 0.91$, $P < 0.001$). In addition to accounting for phylogeny in the db-RDA, we also tested for the independent effect of ET by conducting variance partitioning analysis using the varpart function in vegan. This enabled us to disentangle the individual and shared variation explained by phylogeny and ET. To visualize overall variation in protein-family composition relative to fungal phylogeny, we used hierarchical clustering to generate a dendrogram representing overall Bray–Curtis dissimilarity in total Pfam proportions using the hclust function and the unweighted pair group method with the arithmetic mean type of agglomerative clustering. This visualization technique allowed us to depict variation in Pfam dissimilarity alongside the fungal phylogeny. We then performed indicator species analysis with the multipatt function from the package indicspecies (De Cáceres *et al.*, 2010) to identify Pfams involved in decomposition and N cycling that had significantly higher proportions in genomes of specific ETs.

To examine whether ETs were more abundant on roots vs in the surrounding soil, we plotted relative abundances of ETs across the 50 paired plots against a 1 : 1 line and used *t*-tests to compare differences (see map of sites in Fig. S2). The original analysis of root tip sampling was conducted across years and seasons (van der Linde *et al.*, 2018). While the sampling time between root and soil collection is not constant in our study, we do not expect cross-year variation within the sampling window to be a significant issue. Earlier work has demonstrated that fungal community turnover is relatively low during time periods like the temporal window separating the root and soil sampling in our study (Averill *et al.*, 2019). However, this temporal mismatch is a limitation of the experimental design that often arises from large-scale, synthesis-based research. We also correlated the relative abundances of ETs with each other, including soil pathogen and saprotroph relative abundances as additional covariables. We included these groups because EMF ETs interact with both guilds in a competitive manner for N acquisition (Averill & Hawkes, 2016) and in displacing pathogens (Buscot *et al.*, 1992). The root dataset only contained data on EMF because it was

generated by picking EMF root tips and using Sanger sequencing, so we independently correlated the relative abundance of root ETs with the proportion of soil pathogens and saprotrophs. The root and soil datasets are independent because they were not sequenced together, making these co-occurrence patterns more powerful than the proportional soil comparisons.

Differences in ET relative abundances between dominant tree leaf types (needle vs broadleaf) were analyzed using nonparametric Kruskal–Wallis rank sum tests to overcome issues of heteroscedasticity. We also tested whether EMF ET community composition differed between dominant tree leaf types using PERMANOVA and the adonis2 function in VEGAN.

Relative abundances of ETs were then modeled using partial least squares regression (PLSR) and the plsr function in the PLS package (Liland *et al.*, 2026). PLSR was used in order to include covariables that may be collinear and because a large number of covariables were used to predict ET relative abundances: foliar N levels, foliar P : N ratios to account for the fact that there is high anthropogenic N addition in these historically N limited forests (Etzold *et al.*, 2020), forest productivity, stand age, dominant tree leaf type, soil N content, soil pH, soil clay content, N deposition, MAT, MAP, the interaction between foliar nutrients and tree leaf type, and the interactions between forest productivity and tree leaf type. PLSR works well for ecological data with collinear predictor variables and if there are many predictor variables (Akarachantachote *et al.*, 2014). PLSR models were fit using the orthogonal scores algorithm, and cross-validation was based on 10 segments, randomly selected from the dataset using the 'CV' flag. All predictor variables were scaled to directly compare effect sizes. Variables were log-transformed when necessary to improve model fit, which applied to forest productivity, foliar N, and foliar P : N ratio. Model fits were visualized using the validationplot function.

To compare the same set of predictor variables across every ET, full models containing every predictor variable were first generated for each ET. To identify the most important predictors within the full model, we used backward selection, removing variables with variable importance in projection (VIP) values < 0.8 in the first 1–3 PLS components, with the final component number selected according to the lowest mean-squared error of prediction. VIP values between 0.8 and 1 indicate relatively strong explanatory power and VIP values > 1 have very strong explicative power. VIP values can be ranked to compare effect sizes of different predictors, describing the relative importance of variables to models. In reduced models containing predictors with VIP values > 0.8, we used cross-validation to train models for prediction. Predicted values were used to calculate the root mean square error. This is the average deviation between predicted vs observed relative abundance, and it was used to evaluate model fit alongside the R^2 value.

To visualize patterns among environmental predictors selected in reduced models and relative abundances of ETs, we used Pearson's correlations to obtain correlation coefficients and presented them in heatmaps. Directional changes are less clear from our PLSR results because coefficients are specific to each PLS component. Our complementary approach can therefore be used to show overall positive or negative associations across environmental predictors and ETs but is not very useful for predicting effect sizes because they do not account for other covariables. Because the dominant tree leaf type was accounted for in the combined model but could not be accounted for in individual models, we ran correlations on each tree leaf type separately. Only root data were used for this analysis to keep consistent with PLSR modeling. We only show correlation strength of selected predictors that were retained in our PLSR models after backward selection regardless of the statistical significance of individual Pearson's correlations. Finally, we grouped ETs by their correlation coefficients for each tree leaf type separately by calculating Euclidean distances from the correlation matrices using the vegdist function (Oksanen *et al.*, 2013), and then using hierarchical clustering with ward-D agglomeration in the hclust function.

Results and Discussion

Exploration types harbor unique genomic signatures

Ectomycorrhizal fungal ETs differed significantly in the composition of protein family domains (Pfams) encoded in their genomes. ET had a significant effect on total Pfam composition ($P = 0.001$), independent of genome size and overall gene number, and even after accounting for fungal phylogeny (Table S4). Pfams associated with decomposition and N cycling also differed significantly across ETs (Fig. 3a–c; $P = 0.001$ for both) and remained significant after accounting for phylogeny, genome size, and gene number, with low multicollinearity across models (VIF < 2). Variance partitioning analyses further showed that ETs independently explained 23–26% of the variation in Pfam composition after accounting for phylogeny (Fig. S3). Multiple comparisons indicated that not all ETs were distinct in decomposition (C vs SD) or N cycling (C vs MD-F; MD-F vs LD) Pfams. Phylogenetic distances among taxa also differed across ETs, with the lowest distances within the MD-F type and the greatest within the LD and SD types (Fig. S4). For ETs distributed across phyla, including the SD and C-types (Fig. 2), Pfam composition diverged between Ascomycota and Basidiomycota, suggesting functional divergence among low-biomass-forming ETs at the phylum level. Consistent with this, earlier studies show that C and SD Ascomycota form thinner mantles (Agerer, 2001), have lower carbon demands from host plants (Fernandez *et al.*, 2017), and tolerate drier and warmer conditions (Allison & Treseder, 2008; Geml *et al.*, 2015; Fernandez *et al.*, 2017). By contrast, the MD-F ET spanned a relatively short phylogenetic distance, being represented by few species ($n = 6$), yet including phylogenetically distant lineages such as *Cortinarius* and *Piloderma* (Fig. 2). Despite their divergent evolutionary origins, MD-F taxa shared more similar decomposition and N-cycling-related protein families than more closely related taxa from the SD and MD-S ETs (Fig. 3c). Together, these results indicate that while EMF Pfams exhibit phylogenetic signal, ETs explain unique and biologically meaningful variation in Pfam composition, demonstrating that despite plasticity in ETs and potential classification uncertainty (Box 1), this trait has a distinguishable genomic signature.

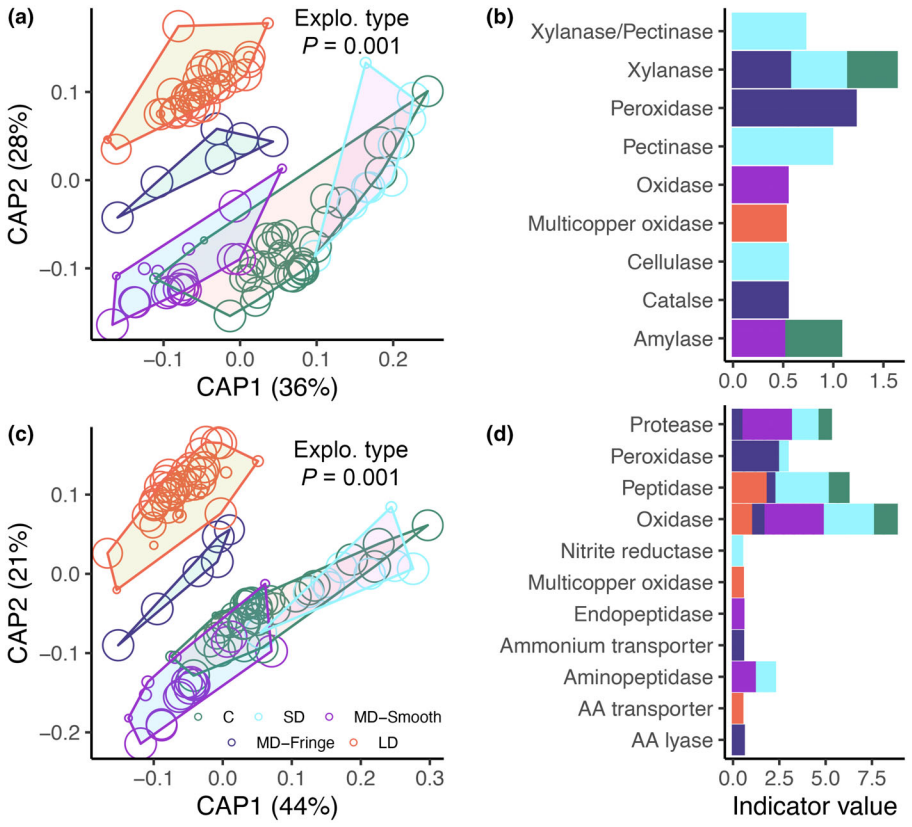

Fig. 3 Genomic variation in exploration type. Variation in protein families encoding enzymes for decomposition of plant biomass (a). Each point represents individual fungal genomes with ranges across exploration types delimited by polygons. Point size represents the mean phylogenetic distance of a taxon. This visualization is included to show that there is a range to phylogenetic distance captured within each exploration type (ET), with some ETs spanning large phylogenetic ranges (i.e. LD, MD-S, SD) and others covering much shorter phylogenetic distances (i.e. MD-F). Significant indicator protein families involved in decomposition (b). Values show the indicator value of each protein family. Variation in protein families encoding for nitrogen cycling (c) and associated indicator protein families (d). *P*-values for the effect of exploration type are shown (a, c) from the db-RDA. See Supporting Information Table S3 for db-RDA statistical results, including multiple comparisons. Abbreviations are for medium-distance fringe (MD-F) and MD-S (MD-S), long-distance (LD), short-distance coarse (SD-C) and SD delicate (SD-D), and contact (C) type exploration types.

EMF ETs have unique indicator Pfams for decomposition and N cycling. Pfams involved in xylan, pectin, and cellulose decay were significant indicators of the SD type (Fig. 1c), while C-type EMF had higher relative numbers of xylan and amylase encoding Pfams. MD-S indicators included oxidases and amylases, while MD-F had the greatest number of peroxidases and catalases. Multicopper oxidases were uniquely associated with the LD type. From these patterns, the capacity to depolymerize lignin via peroxidases and multicopper oxidases is greatest in the MD-F and LD types, at least based on currently available fungal genomes. This would provide these fungi with an important advantage in mining N from soil organic matter and explains why sporophores from these EMF ETs, in many cases, tend to have higher ^{15}N content compared with lower biomass forming ETs (Hobbie & Agerer, 2010; Hobbie & Högberg, 2012). While the SD and C-types were most alike in terms of N cycling genes, the MD-F and LD types exhibited distinct patterns. MD-S and SD had the greatest relative number of proteases and amino peptidases, while MD-F had the greatest relative number of amino acid lyases and ammonium transporters. The LD type had the greatest proportion of AA transporters. This may be an important strategy for transporting amino acids through LD mycelial networks such as rhizomorphs to supply N to host plants (Chalot *et al.*, 2002). Based on these patterns, SD and C-type EMF have similar N use capacities at a genomic level, and in turn, these ETs may have greater niche overlap.

Importantly, these conclusions are based on a limited number of EMF with sequenced genomes (n = 106), and they are based on genomic gene numbers rather than gene expression, so further *in situ* functional investigations are required to assess actual fungal activities in the environment. We also acknowledge that substantial intraspecific genomic variation could shape these results depending on the fungal isolate sequenced within a species. To assess the magnitude of intraspecific Pfam variation, we compared profiles across 12 *Laccaria bicolor* isolates and contrasted this against 12 different *Russula* species and 15 *Suillus* species (Fig. S5), two large and diverse genera. Intraspecific variation was 21–35% lower than intrageneric variation, indicating that although reduced, within-species variability remains considerable. This intraspecific genomic variation may contribute to the plasticity we observe in EMF ET trait expression at the individual species level. Future work should consider how intraspecific variation influences extraradical mycelium development and exploration since it may provide new insights into species-level plasticity.

Biomass productivity and rhizomorph formation explain the distribution of EMF on roots and soil

We expected EMF that produce high mycelial biomass and rhizomorphs to be more abundant in soil than on roots. Our results supported this hypothesis, with high biomass producing groups MD-F, LD, and SD-C having a higher relative abundance in soil compared with roots ($P < 0.001$ for all groups). Curiously, we also observed higher relative abundance in soil for the low biomass but rhizomorph producing group MD-S ($P = 0.001$).

There was no statistically significant difference between soil and roots for C-type EMF ($P = 0.07$), although this type tended to be more dominant on roots than in soils. Finally, SD-D was less dominant in soil compared with roots ($P = 0.02$). Thus, as opposed to biomass production alone, ETs producing intermediate-to-high biomass and rhizomorphs were dominant in soil compared with roots (Fig. 4). It bears considering that soil data may be skewed by DNA obtained from EMF spores and other structures, but there is no known reason to believe this should be biased toward any specific ET. While these results are consistent with earlier studies (Genney *et al.*, 2006; Kjøller, 2006; Parrent & Vilgalys, 2007), this trend has not been consistently observed in studies involving in-growth bags (Jörgensen *et al.*, 2023). We primarily attribute the mixed findings reported in the literature to differences in study scale and high variation in the distribution of EMF ETs across space (see Box 1, for further discussion). Notably, roots and soils were not completely favored by any ET across the studied forests, suggesting that ET habitat preferences are not entirely consistent across the broad geographical scale of our study. Thus, while other studies (Jörgensen *et al.*, 2023) find mixed support for expected ET differentiation between soil and roots relative to their occurrence within in-growth bags, this is potentially due to spatial scale differences across studies (our investigation ranged latitudinally from Sweden to Spain; Fig. S2). Combining two datasets from a continental set of observations enabled us to identify general trends representative of conditions across Europe but which might lead to less accurate stand-level predictions. Based on the distribution of EMF ETs in soil and roots, an intermediate-to-high biomass and rhizomorph production, more than ET alone, can explain patterns of habitat dominance in soil vs roots.

Co-occurrence patterns link biomass production to plant pathogens in soil

Patterns of co-occurrence among EMF, saprotrophs, and pathogens varied between soil and root-inhabiting fungal communities. On fine roots, where EMF must compete for access to carbon, MD-F relative abundances negatively covaried with each ET except SD-C and LD explorers (Fig. 5). This was significant for MD-S ($r = -0.31$, $P = 0.0007$) and SD-D ($r = -0.33$, $P = 0.001$) EMF, two groups that produce low hyphal biomass. MD-F were also the only group significantly negatively correlated with plant pathogens on both roots ($r = -0.52$, $P = 0.0001$) and in soil ($r = -0.11$, $P = 0.02$). Comparing root-inhabiting EMF to soil-dwelling pathogens is statistically independent since the data are not proportional to each other. This result is therefore especially interesting because EMF can reduce pathogen infections of host plants (Buscot *et al.*, 1992; Velmala *et al.*, 2018), and research to date has not demonstrated which groups of EMF may confer pathogen protection. Our observational results hint at the idea that MD-F EMF may be involved in suppressing plant pathogens that live in the soil. Dense aggregations of MD-F hyphae, particularly for cortinaroid EMF, are known

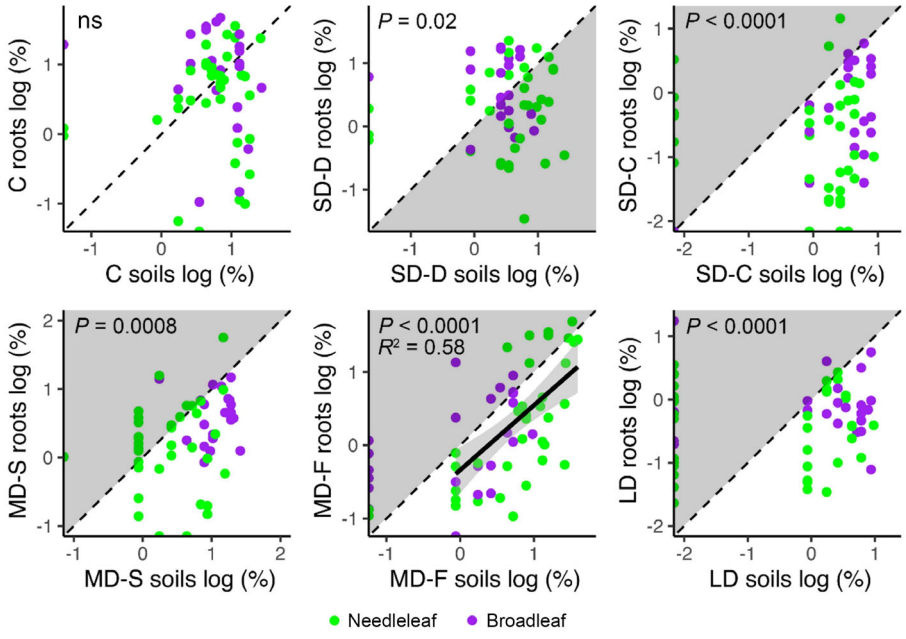

Fig. 4 Correlations between exploration type relative abundances on living roots and in soils. Only MD-F relative abundances on roots and in soil were significantly correlated. To compare whether groups are more dominant in roots vs soils, 1 : 1 lines are plotted. Points to the right of the 1 : 1 lines suggest greater dominance in soils, and points to the left suggest greater dominance on roots. Unshaded sides represent dominance for a habitat type, or lack thereof for C-type. Plots are shown with respect to needle- vs broadleaf dominance using green and purple colors, respectively, and we include this to show similar trends for both dominant tree leaf types. Note that no values were removed for the analysis, including zeros. *P*-values show statistical differences based on two-sided *t*-tests. Abbreviations are for medium-distance fringe (MD-F) and MD smooth (MD-S), long-distance (LD), short-distance coarse (SD-C) and SD delicate (SD-D), and contact (C) type exploration types. ns, not significant.

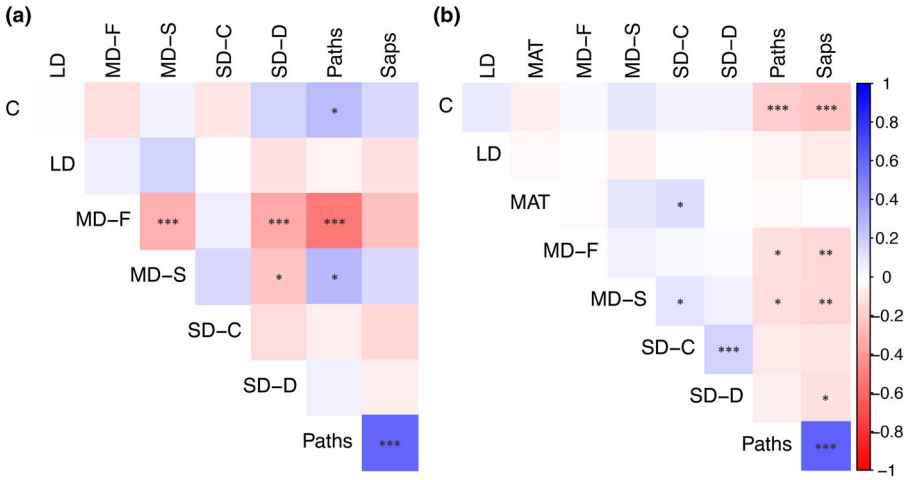

Fig. 5 Patterns of covariation among ectomycorrhizal fungal exploration types on root systems (a) and in soils (b). Colors show Pearson's correlation coefficients and asterisks indicate significance (*, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$). Relative abundances of pathogens and saprotrophs are both from soils since these were not measured on root tips. Exploration type and other trophic mode abbreviations are short for mat-forming (MAT), medium-distance fringe (MD-F) and MD smooth (MD-S), long-distance (LD), short-distance coarse (SD-C) and SD delicate (SD-D), contact (C) type, potentially plant pathogenic (Paths), and saprotrophic (Saps) fungi. Note that MAT-forming ectomycorrhizal fungi were not common enough on root samples to be included.

to outcompete or physically obstruct hyphae of foreign fungi (Agerer, 2006). By contrast, both the C and MD-S types were positively correlated with plant pathogens and may displace fewer pathogens in the soil. We hypothesize that in comparison with low biomass groups such as C and MD-S, MD-F EMF displace plant pathogens in soils either physically via competition or obstruction of roots through high biomass hyphal extension, or an alternative mechanism such as allelochemical production. It will be important for future research to experimentally test these findings since direct causality and directionality cannot be disentangled from our study.

Realized niche differentiation among exploration types

Environmental predictors of the distribution of ETs grouped EMF into broad ecological categories. This analysis is akin to quantifying the 'realized environmental niche' of EMF ETs. Here, dominant tree leaf type (needleleaf vs broadleaf) was a strong predictor of all ET relative abundances except for C-type (Table 1; Fig. 6a). Dominant tree leaf type was linked to neither biomass nor rhizomorph production strategy, with SD-D and MD-F at higher relative abundances in needleleaf dominant forests, and SD-C, MD-S, and LD more common in broadleaf forests (Table 1). It is established that many, but not all, EMF exhibit specificity for either broad- or needleleaf trees (van der Linde *et al.*, 2018). For example, nearly 50% of 300 EMF species exhibited host-specificity in mixed needle-broadleaf forests (Ishida *et al.*, 2007). However, our results show that this degree of specialization also applies when EMF are aggregated at the ET level. For example, there were distinct EMF community compositions within each ET group between broad- and needleleaf forests (Table 1). The greatest differences between dominant tree leaf types were observed for MD-F and LD groups, and the smallest variation was observed for the SD-C and C-type ETs.

While we found grouped responses of ETs to dominant tree leaf type, there were no consistent trends in how ET relative abundances responded to all other predictors (Fig. 6a), and responses to these other predictors differed in correlation strength and directionality between tree leaf types (Fig. 7). Notably, ETs that correlated strongly with foliar nutrients (MD-F, SD-D) exhibited opposite correlational structures between tree leaf types, indicating that polar niches may be formed between needleleaf and broadleaf forests relating to nutrient acquisition. Whether these patterns are driven by differences in EMF composition, differential host plant control over EMF functioning, or other indirect variables remain open questions. Specifically, we observed that MD-F were positively correlated with foliar N in broadleaf but negatively correlated in needleleaf forests, instead favoring higher foliar P : N in needleleaf forests. This is consistent with the hypothesis that high biomass needleleaf-associating EMF are sensitive to N additions (Lilleskov *et al.*, 2011). By contrast, SD-D and LD positively correlated with foliar P : N in broadleaf but showed the opposite in needleleaf forests where they were associated with higher foliar N (Fig. 7). Taken together, these findings suggest that mycorrhizal partnerships between dominant tree leaf types and EMF play a major role in guiding niche partitioning across EMF ETs.

Several indicators suggest that C-type EMF may be more 'generalist' than the other EMF ETs. C-type EMF were equally common in broadleaf and needleleaf forests (Table 1), and we could not predict their distributions as accurately as many other ETs (Table 1), suggesting a degree of generalization that is unique from other EMF. However, patterns between C-type EMF and environmental properties and processes were also stronger in broadleaf vs needleleaf forests, where their relative abundance was negatively correlated with soil pH and clay content (Fig. 7). This demonstrates a particularly tight link between C-type EMF and soil physicochemical properties, consistent with previous work in European oak forests (Suz *et al.*, 2014). Soil clay content was a similarly strong predictor in broadleaf forests as soil pH, potentially because the hydrophilic mycelium of C-type explorers can more efficiently absorb water and inorganic nutrients such as ammonium, which can bind to negatively charged clay surfaces. To that end, C-type EMF were also most common in relatively high N environments and in productive forests (Fig. 7). This lends support to established trends in European forests that C-types are often resilient or positively linked to N deposition (Lilleskov *et al.*, 2011) and is in line with studies showing that trees

Table 1 Variation in relative abundance of ectomycorrhizal exploration types from living roots described by environmental conditions.

			Forest type effects			
Exploration type	PLSR % variation	RMSE %	X^2	P	Br : Ne	Perm. R^2
Contact	27.3	4.05	0.015	0.902	1.01	0.04
Short-distance delicate	27.0	2.27	12.52	< 0.001	0.46	0.07
Short-distance coarse	8.42	3.60	12.91	< 0.001	1.56	0.03
Medium-distance smooth	24.3	6.73	33.23	< 0.001	1.98	0.07
Medium-distance fringe	63.0	2.53	27.41	< 0.001	0.27	0.08
Long-distance	30.7	1.29	4.05	0.044	1.53	0.09

Variation in relative abundance of ectomycorrhizal exploration types from living roots described by environmental conditions, using partial least squares regression (PLSR) on reduced models only containing environmental predictors with VIP values > 0.8 in the first three PLS components. PLSR % variation describes model variation explained by each exploration type, and root mean square error % describes the average deviation between predicted vs observed relative abundance of each exploration type. Results of Kruskall–Wallis rank sum tests show differences in exploration type relative abundances between dominant tree leaf types. The ratio of mean relative abundances in broadleaf to needleleaf forests (Br : Ne) is shown to indicate directionality. A ratio greater than 1 indicates a higher abundance of the exploration type on roots of broadleaf trees, while a ratio < 1 indicates a higher abundance on needleleaf trees. Differences in ectomycorrhizal fungi exploration type community composition between forest types are shown as the coefficient of determination (every comparison was significant at $P < 0.05$).

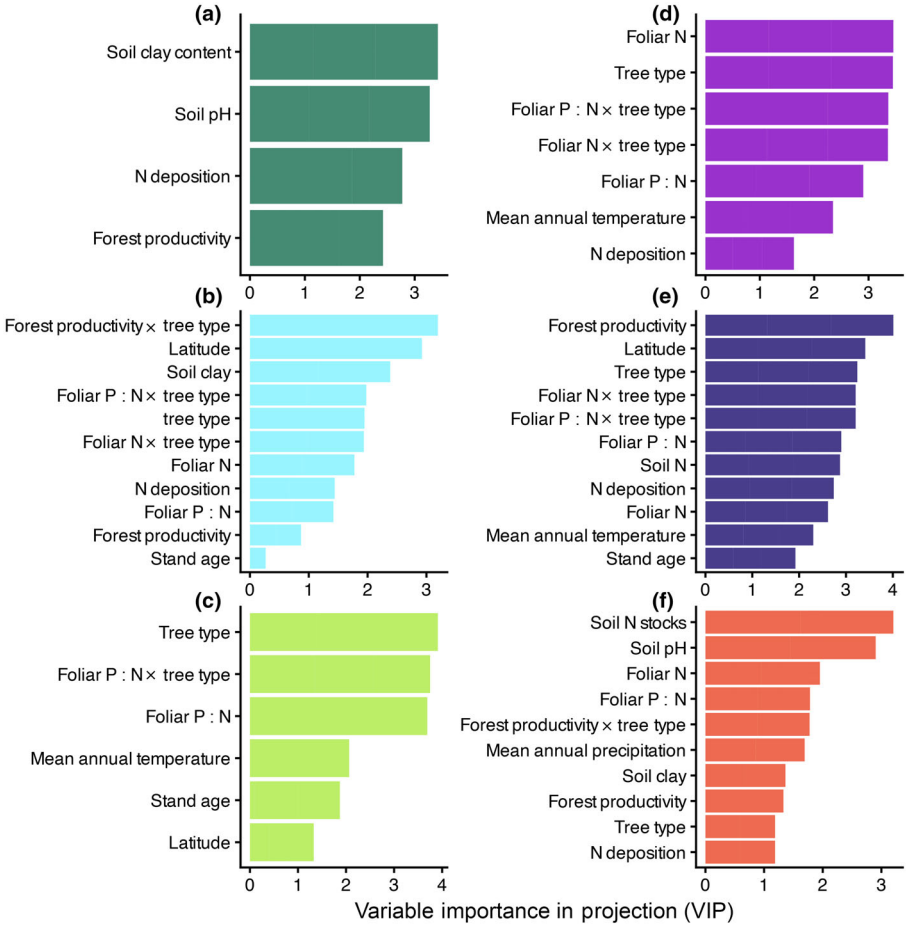

Fig. 6 Patterns among environmental conditions and relative abundances of ectomycorrhizal fungal exploration types in roots across Europe. Summed variable importance in projections (VIP) for components 1–3 of partial least squares regression modeling (the number of components depends on the model). VIP values have been ranked to compare effect sizes of different predictors, describing their importance to variation explained in the models. Separate panels show modeling results for contact (a), short-distance delicate (b), short-distance coarse (c), medium-distance smooth (d), medium-distance fringe (e), and long-distance (f) exploration types.

can adjust N uptake to their demands, thus avoiding N oversupply (Gessler *et al.*, 2004). By contrast, high biomass MD-F was negatively associated with tree growth and N indicators (N stocks and N deposition; Fig. 7). Negative links to N indicators are similar to observations made nearly two decades ago in white spruce (*Picea glauca* (Moench) Voss) forests (Lilleskov *et al.*, 2002). The other high biomass type, LD, showed the same trend with needleleaf tree growth as MD-F; however, LD relative abundances were positively correlated with N deposition in both forest types (Fig. 7). Thus, biomass production amounts may distinguish ET niche suitability between forest types for N indicators, but not forest productivity.

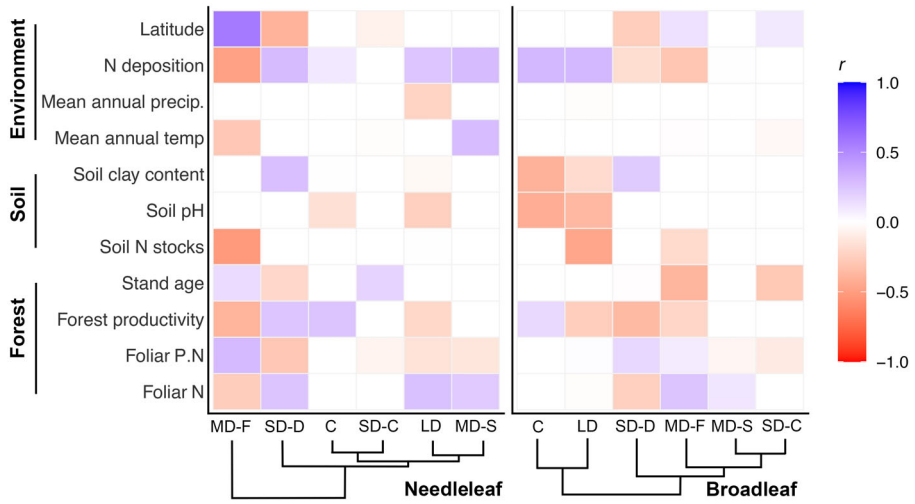

Fig. 7 Visualizations of environmental predictors of variation in relative abundances of ectomycorrhizal fungi exploration types in roots of broadleaf and needleleaf trees across Europe. Heatmap represents Pearson's correlation coefficients between exploration types and predictors. Predictors correlated against exploration types were the same as those selected for partial-least squares regression models (Fig. 6). Stronger correlations are represented by darker shades. Blue = positive and red = negative correlation coefficients. The dendrogram to the left thematically links environmental predictors into tree-related, soil-related, and broader environment-related conditions. The dendrogram below hierarchically clusters exploration types for each tree leaf type using ward-D agglomeration. Note that not all correlations were significant, and we avoid presenting significance here as we intended to identify broad trends and not to analyze direct correlations. Abbreviations are for medium-distance fringe (MD-F) and MD-S (MD-S), long-distance (LD), short-distance coarse (SD-C) and SD-D (SD-D), and contact (C) type exploration types.

Differences in environmental suitability between MD-F and LD EMF ETs may stem from their contrasting decomposition strategies. Our genomic analysis demonstrates that MD-F have greater potential to produce peroxidases, which are suppressed by N deposition (Entwistle *et al.*, 2018), while LD, especially Boletales such as *Pisolithus* and *Scleroderma* (Agerer, 2001; Wu *et al.*, 2022), produce multicopper oxidases that are stimulated by N deposition and aid in lignin breakdown (Freedman & Zak, 2014). These divergent traits help explain observed correlations with ecosystem N metrics and tree growth. MD-F are particularly abundant in needleleaf-dominated forests, where they may limit N uptake under nutrient-poor conditions, reinforcing nutrient limitations rather than alleviating them (Näsholm *et al.*, 2013; Franklin *et al.*, 2014). Their reduced prevalence in high-N environments suggests low tolerance for N enrichment, which has raised concerns under rising N deposition (Lilleskov *et al.*, 2011). Building on earlier hypotheses, we propose that future work should disentangle the roles of MD-F P uptake under relatively low and high N environments. We observed positive correlations between foliar P : N ratios and MD-F relative abundance in both pine and spruce stands (the major representatives of dominant needleleaf forest types; Fig. S6). This stoichiometric imbalance may contribute to reduced tree growth under highly N-limiting conditions and contrasts sharply with patterns observed in LD types. While these relationships are correlative and require experimental validation, they suggest key functional trade-offs among EMF types in responding to and shaping forest nutrient stoichiometry. Ultimately, the roles of EMF in P vs N uptake are still to be fully resolved under low N conditions.

Conclusions and future outlooks

In this study, we explored niche separation in EMF ETs by applying comparative genomics of fully sequenced EMF genomes and pairing DNA-sequenced EMF root and soil communities with forest biotic and abiotic environmental data collected across Europe. We show patterns of separation among EMF ETs from both genomic and biogeographical data types, with a clear progression of similarity from low- to high-biomass ETs. Our first hypothesis that EMF ETs have functionally distinct gene compositions was supported as all ETs showed different protein family encoding gene compositions, and the high biomass MD-F and LD had distinct decomposition and N cycling gene profiles. Our second hypothesis that high biomass ETs would be dominant in soils and low biomass ETs dominant on roots was supported for high biomass ETs, but only low biomass SD-D was dominant on roots, with C-type being equally abundant in both. Finally, our third hypothesis that ETs would show signatures of niche partitioning as a reflection of different links to environmental conditions was only partially supported because it was strongly moderated by dominant tree leaf type (needleleaf vs broadleaf). Our results lend considerable clarity to the value of the trait-classification system for EMF extraradical mycelium that Reinhard Agerer established over two decades ago (Agerer, 2001), and raise intriguing new hypotheses about their environmental niches, capacities to contribute to soil biogeochemical cycles, and potential co-involvement in forest tree growth and nutrient uptake.

We propose that future research focus on the following areas. (1) Comparing EMF ET environmental niches by exposing

culturable isolates or spore-collected taxa to varied microcosm conditions and linking lab-inferred fundamental niches with realized niches from biogeographic data. (2) Comparing actual ET contributions to decomposition, N, and P cycling in microcosms and under *in situ* conditions using metatranscriptomics (*sensu* (Auer *et al.*, 2024)) given gene content investigations only reveal the functional potential of organisms. (3) Testing the differential roles of ETs in pathogen suppression, as our results indicate there may be contrasting feedback among MD-F, MD-S, and C-types with implications for forest tree health independent of nutrient exchange. (4) Experimentally testing the causal effects of different ETs on plant growth and nutrient uptake of contrasting host tree species and leaf types, incorporating ET contributions to host tree development across seasons and growth periods (Xie *et al.*, 2024). (5) Investigating the roles of ETs in P uptake and tree growth within N deposition induced P-limited European forests (*sensu* Zhang *et al.*, 2023) and in forests that remain N limited to test alternative hypotheses about the role of MD-F fungi in responding to or driving N limitation. (6) Lastly, we need continued evaluation of variation in EMF mycelium growth, extent, and exploration using experimentation and direct observation methods, paired with literature reviews and DNA sequencing. In doing so, we can refine and statistically test the efficacy of current trait assignments (Agerer, 2001; Põlme *et al.*, 2020), which may be incomplete for taxa showing extensive morphological plasticity in their extraradical mycelium development (van der Linde *et al.*, 2018; Suz *et al.*, 2021), and in areas of the world where EMF are considerably less studied, such as *Nothofagus* forests of South America, African miombo woodlands, and dipterocarp forests in Southeast Asia. Our results demonstrate that EMF extraradical mycelium attributes differentiate EMF at the genomic and ecological levels, making this an important research area for future development to better understand how variation in EMF communities affects forest functioning and resilience to global change.

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Competing interests

CA is a cofounder of the Society for the Protection of Underground Networks, an organization that advocates for the protection of belowground network forming fungi. CA is also the founder of Funga, a company that facilitates the restoration of belowground fungal biodiversity.

Author contributions

MAA, AZ and TMM conceived the study. TMM and MAA conducted the data analyses. AK generated the phylogenetic tree. MAA, SL, LMS, MIB and FC generated EMF data. TWM, TL, DEAC, PSC, HPV and JH collected and authenticated fungal samples. JMP and LAB extracted nucleic acids. IVG coordinated genome assembly and annotation. TMM and MAA produced a first draft of the manuscript. AZ, FC, LMS, MIB, SVDL, NB, CA, AK, LT, PR, AG, BDV, LC, HM, MW, FJ, PL, AK, MG, GP, BF, MS, MF, PW, VC, RC, GP, AM, LV, MI, HM, VT, NE, AS, FMM, JS, PGK, AK, JMP, ICA, SB, IVG, CJP, SAU, LWZ, OM, IV, TWM, TL, DEAC, PSC, HPV, LAB, and JH reviewed the manuscript and provided feedback; supervision was provided by MAA.

ORCID

Ian C. Anderson https://orcid.org/0000-0002-3507-163X
Mark A. Anthony https://orcid.org/0000-0002-8350-6255
Colin Averill https://orcid.org/0000-0003-4035-7760
Martin I. Bidartondo https://orcid.org/0000-0003-3172-3036
Sara Branco https://orcid.org/0000-0002-9890-933X
Vicent Calatayud https://orcid.org/0000-0002-2792-0988
Roberto Canullo https://orcid.org/0000-0002-9913-6981
Pamela S. Catcheside https://orcid.org/0000-0002-8530-8500
Filipa Cox https://orcid.org/0000-0003-1405-7481
Luc Croisé https://orcid.org/0000-0002-8879-206X
Bruno De Vos https://orcid.org/0000-0001-9523-3453
Marco Ferretti https://orcid.org/0000-0002-8488-0804
Beat Frey https://orcid.org/0000-0002-6391-3574
Arthur Gessler https://orcid.org/0000-0002-1910-9589
Igor V. Grigoriev https://orcid.org/0000-0002-3136-8903
Julia Haska https://orcid.org/0009-0002-1268-3550
Morten Ingerslev https://orcid.org/0000-0003-2790-2904

Frank Jacob https://orcid.org/0009-0001-8623-0653
Peter G. Kennedy https://orcid.org/0000-0003-2615-3892
Annegret Kohler https://orcid.org/0000-0002-9575-9567
Teresa Lebel https://orcid.org/0000-0002-8592-1102
Paweł Lech https://orcid.org/0000-0003-2656-3701
Sietse van der Linde https://orcid.org/0000-0002-1255-8963
Thomas M. Mansfield https://orcid.org/0000-0002-1422-1782
Francis M. Martin https://orcid.org/0000-0002-4737-3715
Tom W. May https://orcid.org/0000-0003-2214-4972
Henning Meesenburg https://orcid.org/0000-0002-3035-4737
Otto Miettinen https://orcid.org/0000-0001-7502-710X
Chris J. Pires https://orcid.org/0000-0001-9682-2639
Jonathan M. Plett https://orcid.org/0000-0003-0514-8146
Genoveva Popova https://orcid.org/0009-0001-6008-5910
Pasi Rautio https://orcid.org/0000-0003-0559-7531
Marcus Schaub https://orcid.org/0000-0002-0158-8892
Laura M. Suz https://orcid.org/0000-0003-4742-572X
Leho Tedersoo https://orcid.org/0000-0002-1635-1249
Volkmar Timmermann https://orcid.org/0000-0001-5058-4639
Sarah A. Unruh https://orcid.org/0000-0002-6896-6405
Lars Vesterdal https://orcid.org/0000-0003-0309-3735
Ilya Viner https://orcid.org/0000-0002-5193-7720
Helen P. Vonow https://orcid.org/0000-0002-3507-4101
Markus Wagner https://orcid.org/0009-0006-2600-9304
Peter Waldner https://orcid.org/0000-0001-8982-6156
Lawrence W. Zettler https://orcid.org/0000-0002-1940-8132

Data availability

The data outputs and scripts that support the findings of this study are openly available in the following repository: https://gitlab.com/fungalecology/emf_exploration_type.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Phylogenetic tree of the fungi used in our analysis labeled with the species name and the designation of the genome in the JGI MycoCosm.

Fig. S2 Map of the study plots with overlapping root and soils data on EMF communities.

Fig. S3 Variance partitioning analysis of the independent prediction of exploration type (ET) and its combined variation explained by both phylogeny and ET.

Fig. S4 Density ridgeline plot showing the distribution of phylogenetic distances spanned in the genomic analysis for each exploration type.

Fig. S5 Coefficient of variation in Pfam composition for *Lacarria bicolor* isolates in comparison to different species of *Russula* and *Suillus*.

Fig. S6 Correlations between aboveground forest growth and foliar nutrition and the relative abundance of medium-distance fringe EMF.

Table S1 List of CAZymes involved in plant biomass degradation targeted for our analysis of EMF decomposition gene variation.

Table S2 List of 106 fungal genomes obtained from the JGI MycoCosm used for genomic analyses with assigned ETs.

Table S3 List of the EMF genera in the root and soil datasets and their assigned exploration type.

Table S4 Distance-based redundancy analysis results and multiple comparisons.

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