“Ectomycorrhizal exploration type” could be a functional trait explaining the spatial distribution of tree symbiotic fungi as a function of forest humus forms

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Summary

In European forests, most tree species form symbioses with ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) fungi. The EM fungi are classified into different morphological types based on the development and structure of their extraradical mycelium. These structures could be root extensions that help trees to acquire nutrients. However, the relationship between these morphological traits and functions involved in soil nutrient foraging is still under debate.

We described the composition of mycorrhizal fungal communities under 23 tree species in a wide range of climates and humus forms in Europe and investigated the exploratory types of EM fungi. We assessed the response of this tree extended phenotype to humus forms, as an indicator of the functioning and quality of forest soils. We found a significant relationship between the relative proportion of the two broad categories of EM exploration types (short- or long-distance) and the humus form, showing a greater proportion of long-distance types in the least dynamic soils. As past land-use and host tree species are significant factors structuring fungal communities, we showed this relationship was modulated by host trait (gymnosperms versus angiosperms), soil depth and past land use (farmland or forest).

We propose that this potential functional trait of EM fungi be used in future studies to improve predictive models of forest soil functioning and tree adaptation to environmental nutrient conditions.

Introduction:

For several million years, gymnosperm and angiosperm trees have co-evolved with symbiotic fungal partners, which help trees grow on diverse types of soil and dominate terrestrial ecosystems (Taylor et al., 2009; Augusto et al., 2014). Most tree species form symbioses with either arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi (Brundrett and Tedersoo, 2018), and some fungal partners (notably EM fungi) play a key role in organic matter (OM) foraging and provide nutrients to the trees (Smith & Read, 2010). Fueled by host photosynthetic carbon, these fungi form vast mycelial networks that extend into the soil in search of nutrients and water (Colpeart et al., 1992; Ekblad et al., 2013). Chen et al. (2016) suggested that AM trees produce more roots to improve nutrient foraging, while EM trees produce more mycorrhizal fungal hyphae to optimize this function. In this way, tree together with its associated mycorrhizal fungi can be considered as a meta-organism or the backbone of the holobiont (Lee et al., 2019; Vandenkoornhuyse et al., 2015). For EM symbiosis, it was hypothesized that the extent of extraradical mycelial proliferation in the soil reflects the spatial exploration capacities of fungi (Agerer, 2006; Finlay & Read, 1986). Ectomycorrhizal fungi are, therefore, a root extension of trees, thereby extending the plant's phenotype (Fernandez et al., 2022). Agerer (2001) classified EM mycelial systems according to the patterns of different exploration types of fungal species based on the amounts of emanating hyphae or the presence and differentiation of rhizomorphs, i.e. cord-forming mycelium. Briefly, the ‘contact’ and ‘short-distance’ types produce only few or short and non-aggregated hyphae in the near vicinity of the root tip. Conversely, ‘long-distance’ and ‘medium-distance’ types form long or aggregated cords with extensive mycelia (Agerer, 2001; 2006). Tree phenotypes could be extended to integrate EM fungal traits enhancing plant nutrient acquisition. In certain studies, specific EM fungi, corresponding to characteristic exploration

types, have been found to reflect different strategies of colonization along the soil profile (Anderson et al., 2014; Genney et al., 2006; Pickles and Anderson, 2016). As an example, Kluting et al. (2019) reported that soil microhabitats support functionally distinct fungal communities with respect to trophic mode and growth morphology, with short-distance EM species being most closely associated with organic layers, while mat-forming EM taxa, in particular *Sarcodon* genera, being generally dominant in mineral horizons. Consecutively, these morphological traits of EM fungi have been proposed as functional traits that could link EM hyphal morphologies to different nutrient acquisition capacities (Agerer, 2006; Defrenne et al., 2019; Zanne et al., 2020). Moreover, an increasing number of studies based on functional trait-based approaches have shown that EM fungi have different nutrient foraging strategies (Koide et al., 2014), such as enzymatic activities (Buée et al., 2007; Talbot et al., 2015), nitrogen uptake (Hobbie and Colpaert, 2005; Kranabetter et al., 2015; Lilleskov et al., 2011), hydrophobicity (Unestam and Sun, 1995; Hobbie et al., 2022) or carbohydrate-active enzyme-encoding genes (Štursová et al., 2012; Barbi et al., 2016; Maillard et al., 2018; 2023). Most of these interpretations are based on studies targeting a single forest site or a single tree species, with their own characteristics, such as soil fertility or the specificity of tree cover. Consequently, the validation of the EM exploration type as a functional trait has not yet been validated in a wide range of forest ecosystems bringing together diverse tree species, soil types and climates in the same study.

Forests functioning and productivity result from the interactions between climate, soil properties, tree species and ecosystem management (Baldrian, 2017). As forest soils are often nutrient poor, tree roots and their mycorrhizal partners must forage different soil layers to mobilize sufficient amounts of nutrients (Legout et al., 2020). Both partners can colonize the forest floor made up of slowly decaying plant residues, and take up the organic nutrients

106 released during decomposition (Lindahl and Tunlid, 2015). They can also prospect nutrients
107 available in the mineral profile of the soil, following the alteration of minerals or the
108 decomposition of organic matter at depth. The concept of humus form was proposed by soil
109 scientists as a descriptor of forest soil functioning, bringing together several biological
110 processes taking place at the interface between plants, soil organisms and soil (Paul, 1984;
111 Ponge, 2013). Consequently, some authors proposed the “Humus Index” as a numerical score
112 which could be used as an integrator of ecosystem functioning (Ponge 2002; Ponge and
113 Chevalier, 2006; Zanella et al., 2011). Humus form is based on a morphological description of
114 OM accumulation along the soil profile, in particular through the thickness of the forest floor.
115 In mull humus forms, dead leaves are rapidly degraded at the soil surface and OM is
116 incorporated by fauna into the deeper mineral horizons, ensuring high availability of nutrients
117 for micro-organisms and plant roots. In contrast, in mor or moder, organic matter accumulates
118 in the forest floor, concentrating nutrient resources, such as plant debris, fauna and microbial
119 necromass on the surface. The question of whether the functional diversity of EM fungal
120 communities and their exploration capacities directly depend on humus index has never been
121 investigated across a large range of forest types and humus forms. In some cases, this question
122 is all the more challenging as the past land use has a lasting influence on the soil fertility of
123 recent forests (Dupouey et al., 2002; Dambrine et al., 2007; Koerner et al., 1997; Verheyen et
124 al., 1999).
125 As the diversity of EM fungal communities is strongly influenced by soil, forest cover and
126 climate parameters (Pérez-Izquierdo et al., 2021), it is essential to improve trait-based studies
127 in mycorrhizal ecology. Indeed, study of fungal traits makes it possible to identify convergent
128 functional responses of distinct mycorrhizal fungal communities (Chaudhary et al., 2022). Our
129 main objective was to study how EM exploration types respond to humus forms. Ultimately,

the aim was to test if the exploration types are functional traits of EM fungi related to the spatial distribution of nutrient resources over the soil profile and to the spatial organization of fungal species within the soil. For this purpose, we studied soil EM fungal communities, using a metabarcoding approach, in seven European countries (9 forest sites) with a total of 34 monospecific tree plantations (nested in nine forest sites, each structured as a "common gardens"). Two types of past land-use were compared: five sites had been established on formerly forested soils (hereafter referred to as "ancient forests") and the other four on former agricultural land (i.e. cropland, shrubland or grassland; hereafter "recent forests"). A total of 23 different tree species were studied (nine EM conifers, two AM conifers, eight EM broadleaves and four AM broadleaves), covering a wide range of climates and humus forms providing broad ecological soil categories. First, we tested if past land-use and tree species are significant factors driving the composition of mycorrhizal fungal communities; second, we used exploratory types as fungal traits potentially correlated with the humus index; and third we evaluated the trait response to humus index, distinguishing between ancient and recent forests. Finally, we aim to propose a conceptual framework to explain the spatial distribution of mycorrhizal fungal species by linking the morphological trait of hyphae (as a potential functional trait for organic nutrient foraging) to the humus index, based on two tree phylogenetic and functional types: conifers and broadleaves (representative of gymnosperms and angiosperms).

Materials & Methods

Site characteristics and soil sampling

153 The study was conducted in nine forest sites in Europe along a latitudinal gradient from Greece
154 to Scandinavia (i.e. Finland and Sweden). Within these sites, structured as common gardens,
155 monospecific tree plantations were established at least 30 years ago on ancient forest sites
156 (former forestry lands), or on former agricultural lands (shrubland, grassland or cropland sites)
157 leading to constitute recent forest sites (Table S1). In each site, we studied two to six tree
158 species, which were selected in order to have pure AM-species or pure ECM-species, thus
159 excluding tree species with both AM and ECM symbionts (e.g. *Eucalyptus* species) or N-fixing
160 tree species (i.e. *Alnus* or *Acacia* species). In total, 34 plots were sampled in all the common
161 gardens, corresponding to 23 different tree species: nine coniferous ectomycorrhizal trees,
162 eight broadleaved ectomycorrhizal trees, two coniferous endomycorrhizal trees and four
163 broadleaved endomycorrhizal trees. Because a few tree species were found in several
164 common gardens (Table S1), it resulted in 34 combinations of site-species, which constituted
165 our study design.
166 During sampling, a detailed description of the humus type was carried out using a classification
167 adapted from those of Brêthes et al. (1995) and Zanella et al. (2011), as summarized in Table
168 S2. This *in situ* analysis was subsequently used to define the humus index for each plot (Table
169 S3) according to Ponge & Chevalier (2006). Sampling was carried out between March 2020
170 and July 2020 (Greece, Southern France, Belgium, Central France), and between March 2021
171 and July 2021 (western France, England, Sweden, Finland, Denmark). Each year, the samplings
172 were carried out from the southern sites to the northern sites in order to ensure that the
173 phenological stages of the trees were comparable. In each plot, six bulk soil samples (6.5 cm
174 in diameter) were collected from three depths after removal of the forest floor: 0–10 cm, 10–
175 30 cm and 30–50 cm. In the Sweden and Finland sites, the bedrock was present at 30 or 40
176 cm, preventing us from collecting samples in the 30–50 cm soil layer. The six soil samples were

merged in the field to constitute one representative composite sample. All composites samples were kept at low temperature (using an electric cool box) until being brought in the lab. Then, samples were sieved to 2 mm to remove roots, stones and organic debris, homogenized and sub-sampled for subsequent analysis. Part of the sub-samples was air-dried and used for physical-chemical soil analyses. The remaining sub-samples were quickly transferred at -80°C for molecular analyses. Soil analyses were carried out by the Laboratoire d'Analyse des Sols d'Arras, INRAE, France (data in Table S3). The cation exchange capacity (CEC) was determined according to the cobaltihexamine method. The pH was determined by the water method using a soil/water ratio of 1:5 (w/v). Soil organic carbon (SOC) and total nitrogen (N) contents were measured using a CHN analyzer, after quantification of carbonates. The content in available phosphorus (P) content was determined according to the Olson method (1957).

DNA extraction, PCRs and sequencing

Genomic DNA was extracted using 500 mg of frozen soil samples (n = 163) using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). The manufacturer's instructions were followed with a few modifications. Adapted from Luis et al. (2004), silica-DNA pellets were washed two times with a solution of guanidine thiocyanate (5.5 M, pH 7) to eliminate any inhibitors of polymerase, then the final pellets were resuspended in 1 ml of guanidine thiocyanate, transferred to the SPIN filter and centrifuged, before continuing with the manufacturer's instructions again. Amplification of fungal ITS1 region was performed using the forward ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA) and reverse ITS2 (5'-GCTGCGTTCTTCATCGATGC) primers (Gardes and Bruns, 1993; White et al., 1990).

PCR was carried out in a total volume of 40 µl including 4 µl of template DNA using REDTaq ReadyMix (Sigma-Aldrich, France). DNA extracts were diluted 1:100 to limit the effect of any PCR inhibitors extracted from sol. PCR amplification was run following this program: 5 min at 94°C, 35 cycles of 1 min at 94 °C, 1 min at 52 °C, 1 min at 72°C and a final extension of 10 min at 72°C. PCR barcode amplifications were confirmed using gel electrophoresis. The multiplexing and the Illumina MiSeq sequencing were done by the PGBT platform (Bordeaux, France).

Raw sequences processing was performed using FROGS (Find Rapidly OTU with Galaxy Solution) pipeline (Escudié et al., 2018). Briefly, overlapping reads were merged using PEAR (Magoč et al., 2011), and adapters were removed with cutadapt. Paired-end reads were merged using VSEARCH (Rognes et al., 2016). Reads were then clustered with SWARM (Martin, 2011), chimera were detected with VSEARCH, and singletons and chimera were removed. Taxonomic assignment was performed against UNITE Fungi 8.2 (Abarenkov et al 2010), and OTUs with affiliations (<90% coverage or <80% identity) were filtered out. Finally, the number of sequences was 982 788 for a total of 1,268 fungal OTUs. Minimum number of total reads in any sample was set at 7121, 7% of the samples were excluded, only 138 samples were finally used for subsequent analyses.

Fungal community analysis and exploration type assignation

The main fungal guilds were defined on the basis of taxonomic affiliation (genus level) using the Fungal Traits database (Põlme et al., 2020) with manual curative control. A total of 403 ectomycorrhizal OTUs have been identified, corresponding to 48 different genera (Table 1). Each genus has been assigned to an exploration type according to Agerer (2001; 2006) and the dEEMY database (Agerer and Rambold, 2011). The exploration types can be separated into

two simplified categories based on the length of the mycelium (and presence of rhizomorphs): "short distance" (SD) or "long distance" (LD). The category SD was composed by contact, delicate and coarser short-distance types (*sensu* Agerer 2001). Contact exploration types have few short hyphae while short distance types may have relatively larger emanating hyphae, but no rhizomorphs are formed. The category LD was composed by medium-distance fringe, medium-distance smooth, mat-forming and long-distance exploration types (Agerer, 2001). In detail, medium rhizomorphs can be divided into three subtypes according to their rhizomorphs' characteristics (fringe, smooth and mat-forming types). Long distance types are characterized by rather smooth ectomycorrhizae with few but highly differentiated rhizomorphs (Agerer 2001). To calculate the relative frequencies of the two main exploratory groups (LD and SD) and the corresponding LD/SD ratios, we used the presence and absence of each EM genus, weighted by the number of species identified within each genus.

Data Visualization and statistical analyses

Statistical analyses and data representations were performed using R software (R Core Team, 2022) version 3.15. Nonmetric multidimensional scaling (NMDS, Bray–Curtis distance metric), PERMANOVA (adonis2 function in the VEGAN package in R; Oksanen et al., 2013) and analyses of similarity (ANOSIM) were performed to test the differences in the structure of the microbial communities. The relationship between humus indexes and the proportion of long exploratory type (LD/SD) was performed using Pearson correlation. Comparative analyses of data were calculated using Kruskal-Wallis tests and we applied correction using the methods of Benjamini-Hochberg (Benjamini and Yekutieli, 2001). Data representations were performed using ggplot package (Wickham, 2016).

Availability of data

The data generated for this study have been deposited with the Sequence Read Archive and are available under the bioproject number PRJNA933461.

Results

In total, we identified 463 EM OTUs present in one or more soil layers: 407 in the 0–10 cm layer, 383 in the 10–30 cm layer and 356 in the 30–50 cm layer (Figure 1). Based on the classification of Agerer (2006), the number for the SD exploration category (sum of contact and short distance exploration types) varied between 194 OTUs (for 30–50 cm) and 207 OTUs (for 0–10 cm). The number for the LD exploration category (sum of medium and long distance exploration types with differentiated rhizomorphs) ranged from 162 OTUs (for 30–50 cm) to 199 OTUs (for 0–10 cm). For all the sites studied, we obtained a balanced distribution of LD and SD - i.e. half-half -, in all three soil depths (Figure 1). These 463 OTUs were grouped into 51 genera, classified equally into SD and LD category exploration types: 25 LD, 25 SD and 1 unknown (Table 1). Depending on the site and associated tree species, the EM genera were distributed within different but overlapping ranges of the humus index: 3–8 under coniferous and 2–7 under broadleaves (Table S3). We did not find any EM genera under the six AM tree species, which were characterized by relatively low humus indices: 4–5 under coniferous and 1–4 under broadleaves, with the exception of *Fraxinus americana* on the Belgian site, which corresponded to a humus index of 7 (Figure S1).

Differences in the composition of EM fungal OTUs were visualized using a non-metric multidimensional scale (NMDS), revealing strong clustering by site (Figure 2). The results of the PERMANOVA model testing the effect of different variables on the structures of the EM

fungal communities showed a significant effect ($P<0.05$) of past land-use, as well as the interaction of past land use with the host species (angiosperms or gymnosperms) (Figure 2; Table S4). By independently analyzing data from ancient and recent forests, our results showed a significant effect of the host tree on EM communities ($P<0.05$) in both types of forest: recent and old forests (Table S4).

Whatever the past land-use, the humus indices of conifer and hardwood stands were positively correlated with the LD/SD ratios (Figure 3A). This relationship was significant for the 10–30 cm and 30–50 cm layers. Although broadleaves are distributed over a lower humus index range than conifers, they generally have higher LD/SD ratios than conifers. Separate analysis of EM fungal communities in ancient and recent forests revealed that the relationship was no longer significant for the two deepest layers in recent forests (Figure 3B). Conversely, although the number of sites observed was lower, the positive relationship between the humus index and the LD/SD ratio remained significant in the two deepest soil layers for the ancient forest sites (Figure 3C). A comparison of soil analyses between ancient and recent forests revealed significant differences in C/N and CEC, regardless of the horizons studied (Table S5). In addition, we measured a significant negative correlation between the humus index and certain soil fertility parameters such as N and CEC in ancient forests, illustrating the link between low soil fertility and humus form (high humus index) in these sites (Table S6). Interestingly, in recent forests (i.e. soils previously used for agriculture, pasture or shrubland), this relationship is reversed, particularly for nitrogen, since a significant positive correlation is measured between soil N content and humus index.

Discussion

Factors driving the composition of mycorrhizal fungal communities

In this study, we investigated the relationships between environmental factors, mycorrhizal fungal communities and their host partners across 23 different tree species, represented by 34 monospecific stands distributed in nine sites across a European transect. Unsurprisingly, we did not find EM fungi in any of the six AM species, whether conifers (*Cupressus* genus) or hardwoods (*Acer*, *Celtis* and *Fraxinus* genera). In addition, the forest floors of these AM species were mostly characterized by relatively low humus indices, between 1 and 5. Conversely, EM tree species had humus indices between 2 and 9, with the majority of plots above an index of 5. These observations are consistent with the results of previous studies showing faster leaf litter decomposition in AM stands compared with EM stands (Midgley et al., 2010; Phillips et al., 2013). In line with our first hypothesis, we found that EM fungal assemblages were strongly dependent on the host plant trait (angiosperm or gymnosperm), and soil fertility as a consequence of past land-use (ancient or recent forests). These results, reported in previous studies (Moora et al., 2014; van der Linde et al., 2018), highlight the need to consider these factors, and their interactions, as important co-variables when studying the functional diversity of EM fungi and their traits. However, there are still major gaps in our knowledge of changes in fungal diversity with regard to associated functional response. Response traits could explain the suitability of fungi to habitats and their ability to influence ecosystem services (Koide et al., 2014). Here, we used a trait-based approach to improve our understanding of the mechanisms by which EM fungi adapt and coexist in different environments or niches (Lajoie and Kembel, 2019).

Relationships between EM morphological traits and humus forms

Although the richness of EM fungi decreased slightly with soil depth, we found that the proportions of LD and SD exploratory types remained relatively well balanced and comparable in the three soil layers studied (i.e., 50/50 ratio). Based on a gradient of forest plots characterized by humus forms ranging from mesomull to humimor, we demonstrated that this LD/SD balance changed significantly with the humus index, a proxy of soil fertility which is negatively correlated with Ellenberg's fertility indices (Lalanne et al., 2010), and CEC or N (present study). Whereas we had not observed any correlation within the forest floor (data not shown), an ecological niche that can be considered a "pantry", the average proportion of EM fungal OTUs belonging to the LD group increased with humus index from 37.2% (humus index 2) to 55.9% (humus index 8). This strong trend, already apparent in the 0–10 cm layer, was significant in the 10–30 and 30–50 cm layers for both conifer and hardwood stands. These results suggest that EM fungi colonizing deeper soil layers explore a greater distance i) to reach the pool of nutrients that are available in the forest floor (i.e. the pantry), particularly when humus forms have changed from mull to moder; or ii) to explore and reach more effectively dispersed nutrient resources in deeper and less fertile layers. Supporting the first hypothesis, previous studies reported that long-distance and medium-distance exploration types directed their growth to organic resource patches (Cairney, 1992; Hobbie & Agerer, 2010). Consecutively, forest fungi may adapt their local mycelial proliferation and spatial distribution related to the vertical distribution of resources (Rosling et al., 2003; Lindahl et al., 2007). In the topsoil, the proximity of organic nutrient resources may explain why the link between the long exploration type and the humus index was weaker. In a coastal pine forest, Kluting et al. (2019) found that short-distance EM OTUs (e.g. *Tylospora* or *Cenococcum*) are more often associated with organic layers (top soil), while OTUs with a medium- and long-distance mat-forming type of exploration, such as *Sarcodon*, are found in mineral and deeper horizons with

343 little presence of roots. Moreover, Peay et al (2011) found that LD exploration types were
344 more frequent in soils with low root density, while SD exploration types were more frequent
345 in soils featuring a high root density in Bishop pine forests. Jörgensen et al. (2023) conducted
346 a 'cafeteria experiment' (i.e. mesh bags filled with different soil and sand substrates) to
347 monitor the EM fungi foraging patterns by incubating these bags in mature *Picea abies* forests.
348 They observed systematic differences in extraradical mycelium proliferation among genera in
349 different substrates. Consequently, they suggested that exploration types are not consistent
350 predictors of soil foraging, proposing that these variations are related to differences in
351 mycelial longevity and the mobility of targeted resources. The artificial nature of that
352 experiment could explain these results, particularly for the observation of adaptive traits
353 requiring long response times. In addition, we cannot rule out two types of adaptation of EM
354 fungal communities in response to resource availability in the soil: i) changes in species
355 composition to favor certain exploratory types and/or ii) changes in extraradical mycelium
356 proliferation among genera as a function of substrates. Our results support the idea that it is
357 necessary to study ectomycorrhizal species traits in order to understand the response of fungi
358 to their environment and to identify the factors that can modify these traits (i.e. trait
359 response). Nevertheless, our study focused on European forests dominated by EM tree
360 species. The objective of establishing a relationship between EM morphological characteristics
361 and humus forms could be extended to other regions of the globe, but mainly in the holarctic
362 zone. Indeed, Tedersoo et al (2010) have highlighted contrasting distribution patterns of EM
363 fungal lineages around the world. For these reasons, if our interpretations can be applied to
364 the forests of the northern hemisphere which harbor the greatest number of EM lineages,
365 future studies should be carried out for tropical forests with *Dipterocarpaceae* and
366 *Caesalpiniaceae* hosts and austral areas characterized by other host taxa. In addition, vast

forest areas are dominated byAM tree species (Schimann et al., 2017; Davison et al., 2022), requiring the study of other traits.

Trait response of ectomycorrhizal fungi to past land-use and soil fertility

Previous studies have reported that the fertility of forest soils explains fungal diversity and causes shifts in mycorrhizal community with decreasing nutrient availability (Kranabetter et al., 2009; Cox et al., 2010). Interestingly, the positive relationship between the humus index and the proportion of long exploration type was partially disrupted in recent forests that had developed on former agricultural or grassland soils. Unlike tree plantations on formerly forest soil, the proportion of LD exploration types at former agricultural land remains stable, at around 50%, between humus index 3 and humus index 9. In recent forests, soil functioning is still in a transitional phase and humus types, normally based on the morphological description of the distribution of OM along the soil profile, do not reflect the actual location of nutrient resources, as organic matter cycles are understood on a secular scale (Balesdent et al., 2018). Past land-use and some human activities have significant and long-lasting effects on soil fertility, due to the persistence of available nutrient such as phosphorus and nitrogen in deep soil horizons, which also has direct impacts on soil organisms (Compton & Boone, 2000; Jussy et al., 2002; Sciama et al., 2009; Jangid et al., 2011; Fichter et al., 2014), including fungal communities and specific ectomycorrhizal genera (Diedhiou et al., 2009; Kjøller et al., 2012; Klavina et al., 2022; Khokon et al., 2023). In a review, Lilleskov et al. (2019) reported that fungal communities primarily composed of EM exploratory types that make up the SD group were characteristic of nitrogen-rich forests, after atmospheric N deposition. In line with this review, we found higher cation exchange capacities and nitrogen and phosphorus contents in former agricultural soils, which could explain the reduction in LD ectomycorrhizal genera in recent

391 forest soils, even in blocks with a high humus index. In addition, our results indicated that the
392 influence of past land-use had a greater effect on the functional diversity of EM fungal
393 communities in the deepest horizons, i.e. down to a depth of 50 cm. Interestingly, it has been
394 reported that EM abundance corresponding to SD exploration types (e.g. *Tylospora* spp.)
395 increased in more fertile soils of *Picea*-dominated forests (Sterkenburg et al., 2015). Similarly,
396 Guo et al (2021) observed that N fertilization induced strong effects on mycelial growth
397 characteristics in pine stands, corresponding to a shift from LD to SD exploration types.
398
399 *Elements for future studies*
400 Hobbie and Agerer (2010) demonstrated EM fungi with longest exploration types (i.e. long-
401 distance, medium-distance mat and medium distance fringe) were more enriched in ^{15}N than
402 EM fungi with shortest exploration type (i.e. medium-distance smooth, short-distance and
403 contact). Furthermore, the relationship between organic N use and N isotopes has been
404 illustrated by Lilleskov et al. (2002), revealing that the ^{15}N of sporocarps was highest for EM
405 fungi that use proteins, and lowest in non-users of proteins. These last authors suggested that
406 EM fungal species vary in their individual response to soil fertility and in their ability to utilize
407 nitrogen bound in soil organic matter (N-SOM). Our results confirmed the limited role of EM
408 fungi in organic N exploration under fertile conditions (Högberg, 2007; Peter et al., 2001;
409 Vesala et al., 2021), resulting in a reduction in mycelial biomass production, i.e. a lower
410 proportion of LD exploratory types (Wallander and Nylund, 1992; Nilsson et al., 2003; Sims et
411 al., 2007). This general model supports the 'ecological market' theory of ectomycorrhizal
412 symbiosis, which advocates for an increasing carbon investment by trees in ectomycorrhizal
413 biomass (and mycorrhization rate) when soil N bioavailability decreases (Franklin et al., 2014;
414 Vicca et al., 2012), and this even though tree growth is ultimately reduced. This mechanism

415 may explain the stabilization of the “rhizomorph strategy” in high index humus *via* a feedback
416 effect of nitrogen depletion.
417 Because there is a large variation in fungal community structure among regions and tree
418 species, the use of functional traits would make possible to determine common community
419 responses to environmental constraints or variables. The exploratory types could be proposed
420 as adaptive response to fertility and spatial distribution of nutrients in the soils. In turn, this
421 trait response can influence the functioning of the ecosystem (Koide et al., 2014) and notably
422 for soil carbon storage and potentially drought, as recently suggested (Castaño et al., 2023).
423 Here, we were able to establish a spatial distribution model of hyphal exploration types in
424 correlation with the humus index, as a proxy of soil fertility, to predict the foraging patterns
425 of EM fungi along the vertical soil profile (Figure 4). Confirming our hypotheses, hyphal
426 exploration type could be interpreted as a functional trait for soil exploration and foraging of
427 OM-linked nutrient. Indeed, we have shown that, in the deepest horizons, the proportion of
428 long exploratory types was higher when soil fertility was low and humus index high, and thus
429 nutrient resources which were mainly located in the forest floor at a distance from to EM
430 hyphae sampled at depth. These correlations lead to two potentially complementary
431 interpretations: the 'rhizomorphic' strategy in the deep layers makes it possible to reach the
432 reserve of nutrients available at the surface in the forest floor or to facilitate efficient
433 exploration in the deeper and least fertile soil layers. We propose that this adaptive trait of
434 EM fungi to nutrient availability in mineral soil horizons (Figure 4) may be strongly affected by
435 past land-use and potential associated fertilizer inputs. We propose to take better account EM
436 exploration types in future studies of forest fungal ecology, as a strategy of OM foraging and
437 nutrient mobilization, in particular N-SOM. However, it seems important to consider a
438 potential limitation to metabarcoding studies using soil DNA, as the presence of detected taxa

in soil does not guarantee that these fungal species colonize roots and form EM morphotypes. Additionally, as suggested by Jörgensen et al. (2023), there may be different growth rates of extraradical hyphae between exploration types as well as a level of phenotypic plasticity within the same EM genus or exploratory type. We conclude that assessing fungal exploration types as indicators of ecosystem functioning or assessing the response of these potential traits to environmental factors could provide relevant knowledge to improve predictive models of soil carbon storage under different forest management scenarios (e.g. succession, mixed forests, plantation, migration). Therefore, more studies are needed coupling trait ecology approaches with directly quantitative methods, such as metatranscriptomics (Auer et al., 2023) and isotopic analyses (Lilleskov et al., 2002; Pellitier et al., 2021; Maillard et al., 2023).

Acknowledgements

This study was made possible by a large, continent-scale, field campaign. Therefore, we gratefully acknowledge all the persons involved in fieldwork, samples handling, and laboratory analyses: Rémi Borelle, Coralie Chesseron, Jean-Luc Denou, Céline Gire, Catherine Lambrot, Julien Langrand, Tania Maxwell, Sylvie Milin, Alain Mollier, Arnaud Reichard, and Samir Moutama (France); Yasmin Jaber, Stamatis Rafail Tziaferidis, Giorgos Xanthopoulos, Fani Lyrou (Greece); Kris Ceunen (Belgium); Frank Ashwood, Sue Benham, Chris Reynolds, Liz Richardson, Mark Oram, Tom Pettingale and Andrew Ross (England); Ming Yu and Haifeng Zheng (Denmark); Dorothea Zannantonio (Sweden); Raino Lievonen (Finland). We particularly thank Lucie Bon and Mohamed El-Mazlouzi, who help us at organizing and leading the field missions. We also thank Bruno Ringeval and Lucas Auer for their help at collecting global climate data and at performing statistical analyses. F.K. held a PhD fellowship awarded by the Grand Est

Region and the “Agence Nationale de la Recherche” (ANR) as part of the ANR project “CARbon functional Traits and their OptimisatioN” - CARTON - (AAPG2019 CES32). This project was supported by funds obtained from the CARTON project and the ANR as part of the ‘Investissements d’Avenir’ program (ANR-11-LABX-0002-01, Lab of Excellence ARBRE).

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

L.A. initiated and coordinated the ANR “CARTON” project. He selected the studied common gardens and interacted with the scientists in charge of those sites (M.N.F., G.S., C.D., R.B., G.G., A.L., B.Z., A.D.S., K.V., E.V., B.M., L.V., A.N., M.J.G., M.L., A.S.), who provided data, ensured monitoring, and supported the field campaign (hosting, field work, tools, shipments, etc.). M.B., D.D. and F.K. designed this study, embedded in the CARTON project. F.K. carried out the molecular biology experiments, handled data, and performed data analyses (bioinformatics and statistics). M.B. and F.K. analysed and interpreted the results. M.B. wrote the manuscript with the help of L.A., D.D., N.F., F.K., M.R.B., and all other authors.

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