

Article

# Patterns in Ectomycorrhizal Diversity, Community Composition, and Exploration Types in European Beech, Pine, and Spruce Forests

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Abstract: Ectomycorrhizal (EM) fungi are pivotal drivers of ecosystem functioning in temperate and boreal forests. They constitute an important pathway for plant-derived carbon into the soil and facilitate nitrogen and phosphorus acquisition. However, the mechanisms that drive ectomycorrhizal diversity and community composition are still subject to discussion. We investigated patterns in ectomycorrhizal diversity, community composition, and exploration types on root tips in Fagus sylvatica, Picea abies, and Pinus sylvestris stands across Europe. Host tree species is the most important factor shaping the ectomycorrhizal community as well as the distribution of exploration types. Moreover, abiotic factors such as soil properties, N deposition, temperature, and precipitation, were found to significantly influence EM diversity and community composition. A clear differentiation into functional traits by means of exploration types was shown for all ectomycorrhizal communities across the three analyzed tree species. Contact and short-distance exploration types were clearly significantly more abundant than cord- or rhizomorph-forming long-distance exploration types of EM fungi. Medium-distance exploration types were significantly lower in abundance than contact and short-distance types, however they were the most frequent EM taxa and constituted nearly half of the EM community. Furthermore, EM taxa exhibit distinct ecological ranges, and the type of soil exploration seemed to determine whether EM taxa have small or rather big environmental ranges.

**Keywords:** ectomycorrhizal community composition; ectomycorrhizal diversity; exploration types; *Fagus sylvatica; Picea abies; Pinus sylvestris* 

# 1. Introduction

Ectomycorrhizal fungi play a fundamental role in the functioning of boreal and temperate forest ecosystems [1,2]. It has been suggested that up to 75% of phosphorus (P) and 80% of nitrogen (N) acquisition is facilitated by mycorrhizal fungi [3–5]. They constitute up to 40% of the total soil microbial biomass C [6,7] and contribute substantially to autotrophic CO<sub>2</sub> respiration [8]. Several studies have shown that up to 68% of photosynthetically derived C was allocated to EM fungi for growth and respiration [5,9].

EM communities are highly diverse [10]. Diversity is highest in temperate and boreal latitudes [11,12], contrasting with the latitudinal gradient of taxa richness found for most groups of organisms [13]. In temperate and boreal forest ecosystems, the richness and diversity of ectomycorrhizal fungi strongly



contrast with the relatively low number of tree species with which they form symbiosis. This relationship is exemplified in monoculture tree stands, where several studies showed dozens of different ectomycorrhizal taxa over less than 0.1 ha [14,15]. Even single root branches can be colonized by several different taxa [16].

The diversity of EM assemblages also manifests in functional diversity, i.e., EM taxa exhibit differences in functional traits. Those functional traits and their effect on ecosystem functioning have received more and more attention in EM community ecology [17]. For example, considerable differences among taxa of EM fungi have been shown in terms of nutrient acquisition [18,19] and other functions, such as C costs for maintenance respiration [20]. Moreover, functional differences of EM taxa that follow different strategies by means of development and differentiation of their external mycelium are evident [21,22], such as differences in which form of N is preferentially taken up [23,24], and the rate at which the hyphal mass decomposes [25]. Classification into different exploration types is a widely used parameter to assess functional diversity of EM taxa, as it is also relevant for ecosystem functioning [26–28]. However, knowledge of these functional traits is restricted to dominant EM taxa. Moreover, our understanding about functional traits and exploration type patterns on a community-level, as well as on larger geographic scales, is still poor and requires further attention.

Several studies showed that EM community composition is altered by environmental factors such as temperature [29–31], water availability [32,33], or N deposition [34]. Changes in community composition can also result in shifts in functional traits at the EM community level [29,35,36]. Although it is known that environmental factors affect EM taxa distribution [37,38], to date, little is known if the distribution of EM taxa is directly restricted by environmental parameters or whether the potential ecological range is mainly a reflection of the ecological range of the host tree species.

Due to the distinctive ecological niches of EM fungi [39,40], understanding biogeographic patterns of EM communities is particularly critical to evaluate the effect of future climate change on EM diversity and community composition. So far, there are only a few studies (see e.g., [11,41,42]) examining large-scale biogeographic patterns of EM fungi. Host specificity was found to be the strongest driver of EM community composition on a global scale [11], while mean annual precipitation and temperature were the strongest drivers of EM fungal richness [11]. On their global analyses on EM communities associated with alder (*Alnus* spp.), Põlme et al. [42] showed that phylogenetic relations among alder species was the best predictor of EM community composition, and soil calcium concentrations positively affected EM fungal richness. Across European oak forests, N deposition and edaphic variables, such as soil pH, C:N ratio, and root density, were found to be the strongest drivers of EM richness and evenness [41]. So far, it remains unclear whether these mechanisms also hold true for EM communities and the different exploration types of the most dominant tree species in Europe, namely *Fagus sylvatica* (L.), *Picea abies* (L.) Karst., and *Pinus sylvestris* (L.) [43].

Therefore, this study aims to explore patterns in EM diversity, community composition, and exploration types across these tree species, and to link particular patterns to environmental and host specific controls. For that, we compiled available literature on morphological and molecular based studies of EM fungal communities on root tips in *Fagus sylvatica* (L.), *Picea abies* (L.) Karst., and *Pinus sylvestris* (L.) stands across Europe.

#### 2. Materials and Methods

#### 2.1. Data Collection

We carried out an analysis based on studies investigating EM fungal community composition on root tips of *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* stands in Europe. We searched for relevant literature in Web of Science and Google Scholar using the following search terms: European beech, Norway spruce, Scot's pine, *Fagus sylvatica*, *Picea abies*, *Pinus sylvestris*, ectomycorrhizal diversity, and community structure. Relevant publications were then checked against expert knowledge for appropriateness. To ensure the data sets were comparable, the following prerequisites had to be

fulfilled: (1) identification of EM taxa on root tips using morphotyping or morphotyping followed by molecular identification techniques; (2) EM taxa abundances in each dataset sum up to 100% and no taxa were combined into groups; (3) the area of the sampling sites was between  $10^{-2}$  and  $10^{2}$  ha; (4) the stand was at least 25 years old. A total of 98 sites from 20 different publications satisfied these criteria (*F. sylvatica*, n = 31; *P. abies*, n = 31; *P. sylvestris*, n = 36), extending from Central Sweden to Central Italy (Table S1, Figure 1). As ectomycorrhizas may be composed of one or more fungal organisms we have used the term 'taxa' throughout the text [44].



**Figure 1.** Map of all 98 study sites in Europe; red dots represent *Pinus sylvestris* stands; green dots represent *Fagus sylvatica* stands; yellow dots represent *Picea abies* stands. Map data © 2018 Google.

All studies that identified root tips by molecular methods (18 out of 20 studies) used National Center for Biotechnology Information (NCBI) and UNITE databases to verify their sequence identity likelihood. Morphological identification only was carried out on two of the used studies (see Table S1). However, studies comparing morphotyping only and presorting, followed by molecular identification, have shown that these two techniques produce similar results [45,46]. Where possible, differently denoted taxonomic units from older studies were aligned with current taxonomic denominations. We did not align the sequence identity of unidentified taxa from older studies due to the paucity of metadata. Metadata on climate (mean annual air temperature, MAT; mean annual precipitation, MAP), site (host taxon, host age, soil pH, N deposition) and sampling (root tip counts) characteristics were compiled, and where missing, obtained directly from the authors. If MAT and/or MAP data could not be obtained, they were subsequently taken from Climate-Data.org based on the geographical coordinates of the site. Data on N deposition (NH<sub>4</sub>, NO<sub>3</sub>, total) were obtained from the Coordination Centre for Effects (CCE) and the European Monitoring and Evaluation Programme (EMEP) [47]. N deposition data from the year 2015 were taken, with the data distributed over a  $50 \times 50$  km grid.

#### 2.2. Data Analysis

In order to account for different sampling strategies between the studies (e.g., sampling design, number of replicates), EM abundance data as used for Figure 2 were standardized prior to analysis. For each site, the ectomycorrhizal taxa were set in relation to the most dominant taxa, yielding relative abundance values between 0 and 1. This standardization was not applied for the other analysis.



**Figure 2.** Relative abundances of ectomycorrhizal taxa across 98 stands of *Fagus sylvatica* (n = 31), *Picea abies* (n = 31) and *Pinus sylvestris* (n = 36) in Europe. To account for possible effects of sampling size and design, all ectomycorrhizal taxa were set in relation to the most dominant taxon of each site, yielding relative abundance values between 0 and 1 for each ectomycorrhizal taxon; e.g., a relative abundance of 1 means that it is the most abundant taxon on all the sites where it is present; or an ectomycorrhizal taxon with a relative abundance of 0.2 is on average a fifth as abundant as the most abundant taxon on the respective plots. Error bars indicate standard error (SE) and are only shown for EM taxa present at more than 10 sites.

In order to evaluate community-level patterns by means of an exploration strategy for EM fungi, we used the Information System for Characterization and Determination of Ectomycorrhizae (DEEMY) database [48], as well as two publications [22,49], to categorize the EM taxa associated with *F. sylvatica*, *P. abies*, and *P. sylvestris* into contact (C), short-distance (SD), medium-distance (MD), and long-distance (LD) exploration types (see Table S2). Data were not used in cases of uncertainty or when two or more exploration types were ascribed at both the species or genus level. One-way ANOVA and Post-hoc Tukey tests were used to test for significant differences at the p < 0.05 level between the different exploration types on EM root tips.

Canonical correspondence analysis (CCA) was used to investigate the effects of environmental variables, including host taxon (tree species), stand age, MAT, MAP, nitrogen deposition (total, NH<sub>4</sub>, NO<sub>3</sub>), and pH, on both EM community composition and EM exploration types. The significances of the environmental variables were tested by means of Monte Carlo permutation tests (*n* = 999). A detailed explanation of this multivariate statistical technique can be found in Reference [50]. CCA's on community composition were restricted to EM operational taxonomic units (OTUs), which were identified at the taxa level and that occurred at least at three sites. A total of 39 sites were used for the analyses, including 12 *Fagus sylvatica* sites, 12 *Picea abies* sites, and 15 *Pinus sylvestris* sites. Data were gathered from 11 studies, employing either morphotyping (11 sites) or morphotyping followed by molecular identification techniques (28 sites) to identify the ectomycorrhizal taxa on the sampled

root tips (as indicated in Table S1). A potential bias between these techniques was tested by adding a variable "identification method" to the CCA. However, no significant effect of the identification method on EM community composition was determined (p = 0.175). Forms of N deposition were analyzed separately and only NO<sub>3</sub> deposition showed a significant effect on the EM community composition. CCA's were conducted in R [51] using the package "vegan" [52]. For reasons of clarity, only a selection of the most abundant EM taxa was written out in Figure 3 The rest of the EM taxa are indicated as "plus".



**Figure 3.** Patterns of variation in (**a**) EM community composition and (**b**) exploration type composition across *F. sylvatica, P. abies*, and *P. sylvestris* stands as explained by pH, mean annual temperature (MAT), mean annual precipitation (MAP), host age, and N deposition (NO<sub>3</sub>) using canonical correspondence analysis (CCA). Small dots represent different plots and large dots represent site averages. Colored dots represent the different sites (green, *F. sylvatica*; yellow, *P. abies*; red, *P. sylvestris*), while grey "plus"-symbols represent different EM taxa. A selection of the most abundant taxa is highlighted with their scientific names, while the rest of the taxa are indicated with a "plus"-symbol. Abbreviations of exploration types: unknown type (UK), contact type (C), short distance type (SD), medium distance type (MD), long distance type (LD). CCA axes show the explained variations in brackets.

Environmental ranges of EM taxa associated with *F. sylvatica, P. abies,* and *P. sylvestris* were identified using the data on host age, MAT, MAP, N deposition, and soil pH. In order to increase the informative value, we only considered EM taxa that occurred at a minimum of five plots and full data on all environmental parameters were available. The minimum and maximum values of the environmental parameters of an EM taxa associated with the three tree species in Europe were used to define an environmental range within the geographical occurrence of the sites within the data set.

Site-level taxa richness was evaluated by means of Simpson's diversity (Simpson's D) and evenness measures [53]. One-way ANOVA was used to test for tree species effects on Simpson's diversity and evenness measures. Regression analysis was used to test for significant relationships between Simpson's D and the following environmental variables: stand age, soil pH, MAT, MAP, and N deposition. The following functions were tested: linear, Gaussian, and logarithmic; and the best model was selected according to the highest  $R^2$  and the lowest *p* value.

#### 3. Results

# 3.1. Patterns in Frequency and Abundance of EM Taxa

Across all 98 sites of *F. sylvatica*, *P. sylvestris*, and *P. abies*, a total of 664 different EM denominations were found, of which 269 (41%) and 177 (27%) were identified to the taxa and genus level, respectively, and 218 (33%) were unidentified (Table S3). The highest number of EM nominations (253) was found in *P. abies*. However, only 84 nominations were identified as taxon, leaving the majority unidentified. In contrast, EM fungal taxa associated with *P. sylvestris* exhibited the lowest taxa richness but the highest level of identification. Only 19 nominations were not identified as taxa. The EM fungal communities of *F. sylvatica* had 240 different EM nominations, of which the vast majority were identified to either taxon or genus level.

Across all sites, the majority of EM taxa (82% in *Fagus*, 51% in *Pinus*, and 66% in *Picea*) occurred solely on one or two sites (Figure 2). The relative abundance of taxa at these sites varied strongly, and over 60% of the EM taxa had a relative abundance less than 0.1, meaning that their abundance was less than a tenth of the taxa with the highest abundance on their respective site. The two most dominant taxa were *Russula ochroleuca* and *Cenococcum geophilum*, both of them considered multi-host taxa, and occurred on a large number of sites with a high relative abundance. There were EM taxa that had a low site frequency but a high relative abundance at the site on which they occurred. An example for such a taxon was *Hebeloma sinapizans* in *F. sylvatica* stands, which occurred only at one site. However, at that site they were the most abundant taxa colonizing  $\approx 12\%$  of the total root tips. Other examples were *Russula pectinatoides* and *Piceirhiza sulfo-incrustata* in *P. sylvestris* stands. Both occurred only at one site but had an abundance of 44% and 39%, respectively. A few EM taxa, such as *Piloderma croceum* or *Tylospora fibrillosa*, showed a highly variable abundance across sites (see Table S1 for detailed information).

# 3.2. Patterns in Soil Exploration of EM Fungi

In terms of abundance, contact (C) and short distance (SD) exploration types showed a higher mean abundance as compared to medium distance (MD) and cord- or rhizomorph-forming long distance (LD) exploration types of EM fungi across all three tree species (Figure 4a). In *F. sylvatica* and *P. sylvestris* stands, the mean abundance of C exploration types was  $11.4 \pm 1.7\%$  and  $12.1 \pm 2.3\%$ , respectively, and thereby significantly higher than any other exploration type. In *P. abies* stands, SD exploration types with an abundance of  $11.5 \pm 1.6\%$  were the most abundant, and significantly different to the MD and LD exploration types.

MD exploration types exhibited a rather low mean abundance of  $4.7 \pm 0.6\%$ ,  $5.9 \pm 0.7\%$  and  $5.0 \pm 0.4\%$  in *F. sylvatica*, *P. abies* and *P. sylvestris* stands, respectively, yet the majority of all EM taxa that could be categorized into exploration types were MD exploration types, with 43.1%, 45.8%, and 50.8% in *F. sylvatica*, *P. abies*, and *P. sylvestris* stands, respectively (Figure 4b). Although C exploration types had the highest mean abundance of all exploration types in the *F. sylvatica* and *P. sylvestris* stands (Figure 4a), this resulted from only a small number of taxa (Figure 4b), being 17.6% of all taxa in *F. sylvatica*, and only 8.5% of the taxa of the EM community in *P. sylvestris* stands. In the *P. abies* stands, SD exploration types had the highest abundance, and constituted on average 30.2% of all EM taxa found in the community. As for the LD exploration types, low mean abundances of  $4.8 \pm 1.0\%$ ,  $3.4 \pm 1.0\%$  and  $4.0 \pm 0.5\%$  in *F. sylvatica*, *P. abies*, and *P. sylvestris* stands, respectively, were recorded as well as low taxa numbers within the EM community, with 11.3%, 6.9%, and 16.3%, respectively.



**Figure 4.** (a) Mean abundance of EM exploration types associated with *F. sylvatica, P. abies,* and *P. sylvestris.* Bars represent mean abundance of each respective exploration type  $\pm$  1 SE. Different letters above the bars indicate significant differences between exploration types within each tree species. \* *p* < 0.05; \*\* *p* < 0.01. (b) Number of EM taxa within each exploration type associated with *F. sylvatica, P. abies,* and *P. sylvestris.* EM taxa proportions of 14.5%, 37.0%, and 20.4% associated with *F. sylvatica, P. abies,* and *P. sylvestris,* respectively, could not be adequately categorized into one of the four EM exploration types.

#### 3.3. Environmental Drivers of EM Communities and Exploration Types of EM Fungi

Environmental variables explained 36% of the total variation (constrained variation) in the EM community composition as assessed by CCA (Figure 3a, Table S4). CCA axis 1 (CCA1) and CCA axis 2 (CCA2) explained 8.4% and 7.5% of the variation, respectively. Host tree species (p < 0.001), soil pH (p < 0.001), MAT (p < 0.001), and NO<sub>3</sub> deposition (p = 0.012) were detected to be significant variables (Table S4). A total of 36% of the variation in EM exploration types was explained by environmental variables. CCA1 and CCA2 explained 26.2% and 9.9% of the variation, respectively. Soil pH (p = 0.002), MAT (p = 0.041), and host taxon (p = 0.016) were detected to be significant variables (Figure 3b, Table S4).

# 3.4. Environmental Ranges of EM Fungal Taxa

EM taxa and exploration types varied in their range within each environmental parameter (Figure 5b). By trend, abundant multi-host EM taxa, such as *Cenococcum geophilum, Russula ochroleuca, Tylospora fibrillosa, or Lactarius rufus,* showed broad ranges for all the environmental parameters examined. These four taxa are considered to be contact and short-distance exploration types. However, broad environmental ranges were also found for low abundant multi-host taxa such as *Thelephora terrestris,* which is considered to be a medium-distance exploration type. On the other hand, the frequently occurring taxon *Xerocomus badius,* a long-distance exploration EM type, exhibited a low mean abundance and was restricted to plots with low precipitation. Abundant EM taxa that were restricted to a single host were found to exhibit confined ecological ranges for at least one of our examined parameters. Two examples were the contact exploration type *Piceirhiza gelatinosa,* which seemed to be restricted by MAP, and *Lactarius subdulcis,* which was found in confined ranges for pH, MAT, and N deposition. Low abundant single-host EM taxa such as *Scleroderma citrinum,* which belongs to the long-distance exploration types, showed narrower environmental ranges for both MAT and MAP, as they were solely found on warm and dry sites (Figure 5b).

	abundance (%)	pH	MAT (°C)	MAP (mm)	stand age (y)	N dep (mg. m <sup>-2</sup> )
	0.1 1 10	2 4 6	2 4 6 8 10	1000 2000 3000	50 100 150 200	1000 2000 3000
Hydnotrya tulasnei (6; Fs Ps)		-	⊢4 -			1
Paxutus involutus (6; LD; Ps)	1 1		<b>⊢</b> ∎1 -	•		
Scieroderina citrinum (6; 1.1); Ps)	] 1					
Inelephora terrestris (13; MD; 1 s Pa Ps)	] ]	· · · · ·	· · · · · ·			
Russula jellea (5; Fs)	] ]					
Lactarius paliaus (7; Fs)	]					
r seuaotomentella tristis (10; SD; PS)	],, ]			·		
Devotorius duatus (17; LD; Fs Pa Ps)						
Fugirniza oleijera (6; F5)	], []]					]
Eupnomyces muricatus (12; 5D; FS PS)	] _ ]					
Elanhamuara municatura (10; MD; FS)	] []					
Lactarius rujus (20; Pa Ps)						
Lastanius (14; 5D; FS Pa PS)	]]					
Elashannasa anaulatus (14: CD: E- D- D-)						
Lectanius subduleis (24, Es)	] []					
Concentration (55, C) Fa Pa Pa)						
Piceirniza geiarmosa (11; C; Pa)						
Tytospora fibrituosa (16; SD; Fs Pa Ps)						
	1 1					
Pinus sylvestris (Ps)		⊢▲───┤ -	▲-  -	⊢▲	<b>⊢</b>	- <b>⊢</b> ▲ — — I
Picea abies (Pa)	1 1	⊢▲	<b>⊢</b> ▲ · ·	<b>⊢</b> ▲──   -		-   <b>-</b>
Fagus sylvatica (Fs)		⊢▲	<b>⊢</b> ▲→  -	+▲  -		
a						

**Figure 5.** (a) Environmental ranges provided by each tree species; (b) environmental range of EM taxa associated with *F. sylvatica*, *P. abies*, and *P. sylvestris* found on at least five sites; numbers in parentheses indicates frequency on number of sites (from a total of 98 sites); letters in parentheses indicate the exploration type (*C*, contact type; *SD*, short-distance type; *MD*, medium-distance type; *LD*, long distance type) and the different host tree species (*Fs*, Fagus sylvatica; *Ps*, Pinus sylvestris; *Pa*, Picea abies). Triangles represent the median, and error bars represent minimum and maximum environmental range.

# 3.5. EM Taxa Richness and Evenness

The mean Simpson's Diversity Index of the EM communities were 7.76  $\pm$  0.95, 6.81  $\pm$  0.51, and 7.57  $\pm$  0.58 for *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* forests, respectively, and no significant differences between the tree species were detected (*F* = 0.499, *p* = 0.609). The mean Simpson's Evenness Index of the EM communities were 0.016  $\pm$  0.002, 0.015  $\pm$  0.003, and 0.017  $\pm$  0.006 for the *F. sylvatica*, *P. abies*, and *P. sylvestris* stands, respectively, and no significant differences between the tree species were detected (*F* = 0.499, *p* = 0.609).

Linear regression analyses revealed no effect of MAT, MAP, or total N deposition on EM diversity by means of Simpson's Diversity Index across all three tree species (Table S5), but diversity was found to be highest at an intermediate tree stand age (p = 0.014) and soil pH of 6.5 (p = 0.017) (Figure 6). Stand age was negatively correlated to Simpson's evenness measure (F = 3.152,  $R^2 = -0.249$ ; p = 0.039) (Table S5), indicating that EM fungi become more evenly distributed with increasing stand age. Otherwise, no significant correlation was found for other environmental variables and taxa evenness by means of Simpson's Evenness Index (Table S5).



**Figure 6.** Regression between Simpson's diversity Index (Simpson's D) and (**a**) stand age, (**b**) mean annual temperature, (**c**) N deposition, (**d**) pH, and (**e**) mean annual precipitation across *F. sylvatica*, *P. abies*, and *P. sylvestris* stands in Europe; solid lines denote Gaussian (**a**,**d**) and linear (**b**,**c**,**e**) regression lines.

# 4. Discussion

#### 4.1. Environmental Drivers of EM Communities and Exploration Types

In accordance with global-scale studies [11,12], host tree species is the most important factor shaping EM communities in Fagus sylvatica, Picea abies, and Pinus sylvestris forests in Europe (Table S4). The circumstance that tree species appears as an explanatory variable might be an indirect effect of the site conditions where the tree species grow. Superimposed upon the filtering effect of the host tree are edaphic and climatic factors such as soil pH, N deposition, or mean annual temperature. In line with our study, large-scale meta-analyses and experimental manipulation studies provide ample evidence that temperature significantly influences EM diversity and community composition [11,29,30,36,54,55]. Soil pH exhibited a significant effect on both EM diversity and community structure. A similar pattern is evident for both bacterial and fungal communities, where community composition strongly varies with soil pH [12,56], and highest diversity levels were found at soils close to neutral pH [12,57]. The significant effect of pH on EM community composition of Fagus sylvatica, Picea abies, and Pinus sylvestris forests in Europe contrasts with findings from Tedersoo et al. [11], which highlighted that climatic factors, such as mean annual temperature and mean annual precipitation, were the main predictors of EM fungal richness. However, that study might have neglected soil chemical parameters, as pH was found to significantly shape EM community composition [42,58,59]. Moreover, the highest EM diversity was also found for an intermediate stand age, probably due to the fact that late- and early-stage EM fungi (sensu [60]) appear together at stands with an intermediate tree age. Increased N levels due to deposition is known to alter above- and below-ground EM diversity and community composition as well as mycelial growth [34,61–64]. While most studies investigated local and regional scales, the significant N deposition effects on EM communities is also valid on a European scale [41]. We found  $NO_3$  deposition, yet not total N deposition, to be a significant driver of EM diversity and community composition in our dataset. This could be due to the limited quality of

our meta-data on N deposition, which had to be gathered from other sources for most publications. Mean annual precipitation showed no effect on EM diversity and community composition across all three analyzed tree species. This is in contrast to global-scale analyses of EM fungi, or rather fungi in general, where a negative effect of increased precipitation was found [11,12]. This negative effect was ascribed to stress from low oxygen availability in water-saturated soils, which is particularly problematic for tropical soils. However, as our analysis just covered temperate and boreal biomes, and because we had a quite small range in mean annual precipitation. Furthermore, studies that experimentally induced drought showed shifts in the EM community composition [31,65–67], yet the effect on EM diversity was not so clear. For example, no significant effect of drought on EM diversity was found in two studies analyzing *Quercus* spp. [65,67], while drought significantly decreased the EM fungal richness of *Fagus sylvatica*, *Picea abies* [31], and *Pinus edulis* dominated forest systems [66].

Mean annual temperature, pH, and tree species were also found to significantly influence the distribution of the different exploration types across all tree species (Table S4). Within the confines of our dataset, the type of soil exploration seems, at least for the most frequent and dominant EM taxa, to determine whether EM taxa have small or rather big environmental ranges (see Figure 5). Contact or short-distance exploration types of EM fungi had, for the most part, broad environmental ranges. *Cenococcum geophilum*, a short distance exploration type, is reported to be the most frequently detected EM taxon worldwide [68], exhibiting an extremely wide host and habitat range. In our study it was found on 74% of all sites and was the most dominant taxon on every fifth site. Cenococcum geophilum is known for its high pioneer capability [69] and its potential to cope with extreme abiotic conditions [70,71]. In contrast, LD exploration types of EM fungi, such as *Xerocomus* badius, Scleroderma citrinum, or Paxillus involutus, were particularly restricted to sites with high mean annual temperatures and low precipitation. Higher abundance of rhizomorph-forming EM fungi due to warming has been found for terrestrial arctic ecosystems [29,54] and at the boreal-temperate ecotone [72], where compositional shifts were linked to host photosynthetic rates. Therefore, increased photosynthetic rates might allow the tree hosts a higher allocation of C to their fungal symbionts [73], which sustains rhizomorph-forming MD and LD exploration types that are thought to be more carbon demanding than C and SD exploration types [74,75]. Moreover, decreasing precipitation favors EM taxa with long rhizomorphs because of their ability to better explore the soil matrix and, subsequently, to transport water more efficient [76]. There is strong evidence of functional changes in EM communities by means of exploration strategy due to anthropogenic N deposition [23,41]. MD fringe exploration EM types declined drastically, while positive responses were noticed for C, SD, and MD smooth exploration types [63,77,78]. In contrast to those studies, N deposition was not found to be a significant driver of exploration type distribution in our dataset. This could be attributed to the lacking quality of meta-data on N deposition, which were not given in most studies and had to be gathered from other sources. There is evidence that N deposition is inversely correlated with soil pH [41,79]. Therefore, we could see the effect of N deposition indirectly through soil pH, as this variable was found to significantly alter the distribution of EM exploration types. In support of this, other parameters that are also linked to soil pH, such as K, Ca, or Mg concentrations, were also found to significantly affect EM community composition [42,58].

# 4.2. Patterns in Frequency and Abundance of EM Taxa and Exploration Types

Environmental parameters drive EM communities in which rarity is a pronounced phenomenon. There are several aspects that impede our understanding of rare EM taxa: (1) the temporal and spatial dynamics of EM communities [80,81], (2) the spatially patchy and clumped distribution of many smaller genets [82], (3) the fact that detection of rare taxa is strongly coupled to sampling effort [82,83], and (4) the circumstance that taxa with a similar appearance are either not recognized or misidentified [84,85]. Multiple dimensions of rarity must be considered. Not only can EM taxa be rare at the plot-level, the vast majority of EM taxa occurred solely on one or two out of 98 sites.

Though, a taxon may exhibit a high on-site abundance on one or two sites, but a low across-site frequency. Such taxa dominate a niche and competitively exclude other taxa. Yet, the mechanisms behind local dominance but landscape rarity are yet to be discovered. In the case of low across- and on-site abundance, rare EM taxa may either have a high degree of functional complementarity [86], or they are restricted by the selective pressure of the environment, as our analysis suggests. Hence, rare EM taxa might be prone to extinction due to the stronger selective pressure that environmental factors exhibit on them. Those rare taxa might be particularly important to ensure functional stability under changing seasonal or environmental conditions [87,88].

Our community-level analysis of exploration types indicates a dominance of contact and short-distance exploration types over medium- and long-distance types by means of abundance (see Figure 4). Yet, nearly half of all EM taxa in these communities were medium-distance types. Long-distance exploration types, on the other hand, were both low in frequency and abundance. The variation in morphology (rhizomorphic in LD vs. diffuse in C and SD) and biochemistry (hyaline in LD vs. melanine in C and SD) of the different exploration types has implications for ecosystem functioning. For example, long-distance types are able to form common mycelial networks [3]. These types exhibit higher acquisition of organic N [24,26,77,89], increased water withdrawal and nutrient uptake efficiency [28], proportionally higher N and C cycling within the plant-fungus complex, and higher turnover of soil organic matter than C [90]. On the other hand, short-range exploration types (C and SD) preferentially take up N as nitrate and ammonium [24,26,78,89], and they have been found to facilitate humus build-up due to the biochemical nature of the mycelium [91], leading to a high C sequestration in soils. Thus a shift in exploration type composition driven by environmental change will likely affect the C and N cycling of the ecosystem.

#### 4.3. Limitations of the Study

Although we assembled a data set using all available published data that fulfilled our criteria, there are clearly shortcomings. A number of the studies lacked information on sampling design and effort, and it is known that sample number and volume significantly affect estimates of EM diversity [11,82]. Only a small number of publications evaluated their sampling effort by calculating taxa accumulation curves. While abundant EM taxa might still be reliably estimated, the number of less frequent taxa might be disproportionately underestimated, owing to the high taxa richness in EM fungi, their clustered distribution and their temporal and spatial variation [92]. Although we have not detected any effect of sampling effort on EM taxa richness or Simpson's D across all tree species, an earlier examination of *Fagus sylvatica* stands revealed a significant relationship between the number of counted root tips and EM taxa richness [84]. Furthermore, the study sites used in our analyses are spatially unequally distributed across Europe and might not cover the whole environmental range of the host trees, and as many EM taxa can also be associated with other tree species, the potential environmental range of EM taxa is probably larger than we could capture with our dataset. Despite these limitations, our results shed light on the structure of EM communities in European forests and the importance of large-scale environmental parameters that, among others, considerably affect the distribution of EM fungi.

#### 5. Conclusions

In this study we show that EM communities in European forests comprise of a small number of abundant taxa and a large number of rare taxa. Contact and short-distance exploration types were highest in abundance, while medium-distance exploration types constituted around half of the EM communities. Long-distance exploration types were both low in frequency and abundance. We furthermore show that both host taxon and environmental parameters significantly shape EM communities and exploration types across European forests. The four most abundant and frequent taxa *Cenococcum geophilum, Piceirhiza gelatinosa, Russula ochroleuca,* and *Tylospora fibrillosa,* all of which are considered contact or short-distance exploring EM taxa, show broad ranges for all the environmental parameters examined. On the other hand, low abundant EM taxa show narrower environmental niche breadths for both MAT and MAP, which seem to limit their occurrence. EM taxa with narrow ecological ranges are particularly threatened by environmental changes. As climatic conditions in Europe are expected to change in the future, shifts in EM community composition are therefore very likely. This might have significant effects on biogeochemical processes and C sequestration in European forest ecosystems.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1999-4907/9/8/445/s1, Table S1: Abundance of EM taxa in *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* from 98 sites including site characteristics and further information; Table S2: Data sheet for EM exploration types on root tips in *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* at a sylvatica, *Picea abies*, and *Pinus sylvestris* from 98 sites including site characteristics and *Pinus sylvestris* stands; Table S3: Levels of identification in ectomycorrhizal communities found on root tips of *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris*; Table S4: Results from Canonical Correspondence Analyses (CCA) on ectomycorrhizal (EM) community composition and exploration types of EM fungi. Shown are the total inertia of CCA's and inertia explained by the constrained environmental variables pH, mean annual temperature (MAT), mean annual precipitation (MAP), host age, NO<sub>3</sub> deposition, and host taxon (tree species). Further shown are the eigenvalues of the first and second CCA axes and the significances (*p*-values) of the individual environmental variables as assessed by Monte Carlo permutation tests; Table S5: Regression analysis between environmental parameters (pH; mean annual temperature, MAT; mean annual precipitation, MAP; stand age; N deposition) and Simpson's Diversity Index (Simpson's D), as well as Simpson's Evenness Index (Simpson's E) of the EM communities in *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* stands across Europe.

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