

Article

Ectomycorrhizal Assemblages of Invasive *Quercus rubra* L. and Non-Invasive *Carya* Nutt. Trees under Common Garden Conditions in Europe

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Abstract: Invasive tree species change biodiversity, nutrient cycles, and ecosystem services, and can turn native ecosystems into novel ecosystems determined by invaders. In the acclimatization and invasion of alien tree species, the crucial role is played by ectomycorrhizal (ECM) fungi. We tested ECM fungi associated with *Quercus rubra* and *Carya* trees that are alien to Europe. *Quercus rubra* is among the most invasive tree species in Europe, and the *Carya* species are not considered invasive. Both form ectomycorrhizal symbiosis, and in their native range in North America, coexist in oak-hickory forests. Six study stands were located in Kórnik Arboretum: three for *Q. rubra* and three for *Carya* trees. Ectomycorrhizal fungi were assessed by molecular identification of ECM roots. We identified 73 ECM fungal taxa of 23 ECM phylogenetic lineages. All identified ECM fungi were native to Europe. Similar richness but different composition of ECM taxa were found on *Q. rubra* and *Carya* roots. Phylogenetic lineages /tomentella-thelephora, /russula-lactarius, and /genea-humaria were most abundant on both *Carya* and *Q. rubra* roots. Lineages /tuber-helvella and /entoloma were abundant only on *Carya*, and lineages /pisolithus-scleroderma and /cortinarius were abundant only on *Q. rubra* roots. Analysis of similarities revealed a significant difference in ectomycorrhizal assemblages between invasive *Q. rubra* and non-invasive *Carya*. Highlights: (1) under common garden conditions, ECM taxa richness was similar on *Q. rubra* and *Carya* roots; (2) ECM taxa composition differed between invasive *Q. rubra* and non-invasive *Carya*; (3) high abundance of long-distance exploration type (lineages from Boletales) was on *Q. rubra*; and (4) high abundance of short-distance exploration type (e.g., /tuber-helvella) was on *Carya*.

Keywords: alien species; biological invasions; symbiosis; fungal ecology; exploration types; phylogenetic lineages



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1. Introduction

In Europe, the ongoing climate warming generates unfavorable conditions for numerous native tree species [1–4] and concurrently, more optimal conditions for others, including alien and invasive tree species [1,5]. The biological invasion is one of the most crucial threats to the maintenance of native forest ecosystems [6–8]. Invasive tree species change local and regional biodiversity, nutrient cycles, and ecosystem services, and transform native habitats into novel ecosystems determined by invaders [8–12]. Trees from North America have turned out to be well-adapted to the local climate in Europe, and often have become invasive species in the native forests [6].

The red oak (*Quercus* sect. *Lobatae*, Fagaceae) and hickories (*Carya* spp., Juglandaceae) are large deciduous trees native to humid subtropical and continental zones in the southeast region of North America [13]. In Europe, they have been introduced and planted since the 17th century due to their valuable wood, ornamental qualities, and edible nuts in the case of hickory species [14–16]. *Quercus rubra* has been naturalized throughout most of Western and Central Europe because of its economic productivity as a fast-growing

source of timber [16]. Nowadays *Q. rubra* belongs to the five most invasive and widespread alien tree species in Europe [17]. In native forest ecosystems in Europe, invasive *Q. rubra* reduces the richness and cover of native plant species [16,18–21]. On the other hand, *Carya* species have not been considered invasive in native ecosystems in Europe, despite being well-adapted to the local environment [6,15,22].

The oldest individual red oak and hickory trees in Europe are located in botanical gardens, where non-native trees were planted in the first place [6,14]. Arboreta are dendrological gardens dedicated to the cultivation, collection, and research of trees from different geographical regions. Kórnik Arboretum in Poland as one of the largest and oldest arboreta in Europe contains more than 3000 species and varieties of coniferous and deciduous trees and shrubs on an area of around 0.53 km² [14], thereby creating favorable conditions for the scientific investigations of alien and invasive tree species under the same climatic and similar soil conditions.

We tested ECM symbiotic assemblages of over 100 years old *Quercus rubra* and *Carya* trees grown under common garden conditions in Kórnik Arboretum in Poland. The study aimed to compare ECM assemblages between invasive and non-invasive tree species under common climatic and similar soil conditions and find the potential differences in tested ECM assemblages. In their native range in North America, both *Q. rubra* and *Carya* trees co-exist in oak–hickory forest ecosystems, similar to the European oak–hornbeam forest. *Quercus rubra* belongs to the Fagaceae family, entirely associated with ectomycorrhizal (ECM) fungal symbionts [23]. *Carya* belongs to the relic tree family Juglandaceae, which contains both ectomycorrhizal trees (e.g., *Carya*, *Oreomunnea*, *Alfaropsis*, and *Engelhardtia*) and arbuscular mycorrhizal (AM) trees (e.g., *Juglans* and *Pterocarya*; [24–26]). Our previous studies revealed that *Carya* seedlings can also form associations with arbuscular mycorrhizal fungi [14], similarly to seedlings of *Quercus rubra* [27,28]. However, because ectomycorrhiza is the predominant type of symbiosis for mature *Quercus* and *Carya* trees, and the morphological identification of AM-like structures inside the roots of ECM trees is disputed [29], we tested ECM fungi only. Ectomycorrhiza plays a key role in the proper development and functioning of almost all tree species in the Northern Hemisphere [23,29]. Thus, ECM symbiosis affects the adaptation and naturalization of non-native tree species, and the presence of appropriate ECM fungal species can enable and accelerate the invasion of alien tree species [30–32].

To the best of our knowledge, this was the first study comparing the ECM assemblages of invasive *Q. rubra* and non-invasive *Carya* trees grown together under common garden conditions. In Europe, *Carya* trees form species-rich ECM assemblages reaching up to 19–25 and 20–40 ECM taxa per site for seedlings and mature trees, respectively [14,15]. On the contrary, *Q. rubra* is characterized by a lower richness of ECM symbionts—less than 10 for seedlings and 10–28 ECM taxa per site for mature trees [33,34]. The dominant share of *Cenococcum geophilum* sensu lato, which reaches up to 80–90% of ECM root tips per site [33,34], is considered to be typical for *Q. rubra* [16]. On the roots of *Carya* trees in Europe, *C. geophilum* reached up to 14–22% of ECM root tips per site [14,15]. Thus, we expected to find lower species richness and different species composition and taxonomic and functional diversity of ECM fungi on *Q. rubra* than *Carya* roots.

2. Methods

The study was carried out in the area of Kórnik Arboretum in Poland, in Central Europe (52.2422° N, 17.0926° E). More than 150 years old red oak and hickory trees were tested in six study stands: three for *Q. rubra* and three for *Carya* trees. Each of the three study stands for *Q. rubra* were located with 220–400 m distance to each other. Each of the three study stands for *Carya* were located with 290–400 m distance to each other. Two of the three study stands for *Q. rubra* were located beside two stands for *Carya* (Figure 1). Tested trees belonged to the oldest representatives of selected tree species in Europe (collection found in the years 1845–1874 [14]). The composition of tree species from the surrounding area is presented in Table 1.

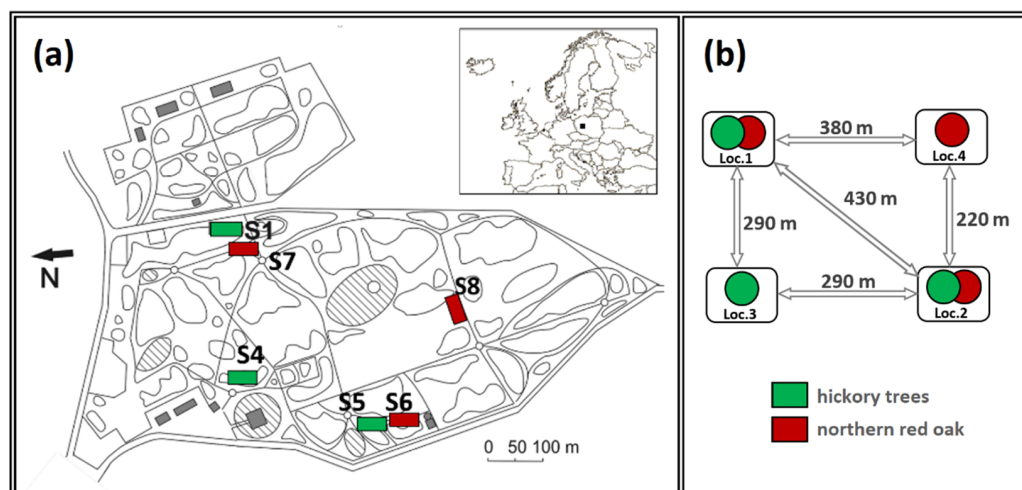


Figure 1. Study stands (a) and the distance between locations 1–4 (b).

Table 1. Tree composition in the surrounding area of tested tree species (Ø 50 m).

Site	Location 1	Location 2	Location 3	Location 4
Stand Type	<i>Carya lacinosa</i>	<i>Quercus rubra</i>	<i>Carya ovata</i>	<i>Quercus rubra</i>
soil pH [H ₂ O]	5.09	4.93	5.82	5.48
			6.40	5.24
Native tree species	ECM	<i>Tilia cordata</i> <i>Carpinus betulus</i> <i>Fagus sylvatica</i>	<i>Tilia cordata</i> <i>Carpinus betulus</i> <i>Fagus sylvatica</i> <i>Quercus robur</i>	<i>Tilia cordata</i> <i>Quercus robur</i> <i>Populus alba</i> <i>Picea abies</i>
	AM	<i>Taxus baccata</i> <i>Acer campestre</i> <i>Acer platanoides</i>	<i>Taxus baccata</i> <i>Acer campestre</i> <i>Acer platanoides</i>	<i>Taxus baccata</i> <i>Ulmus laevis</i>
Alien tree species	ECM	<i>Picea pungens</i>	<i>Abies homolepis</i> <i>Castanea sativa</i>	<i>Picea obovate</i> <i>Abies sibirica</i>
	AM	<i>Aesculus hippocastanum</i> <i>Thuja</i> spp. <i>Pterocarya fraxinifolia</i> <i>Juglans cinerea</i>	<i>Liriodendron tulipifera</i> <i>Magnolia acuminata</i> <i>Platanus × hispanica</i>	<i>Thuja</i> spp. <i>Fraxinus chinensis</i> <i>Gleditsia triacanthos</i>

The diversity of ECM fungal symbionts associated with trees was assessed by the molecular identification of ECM roots. Root samples (fine roots) were collected between August and October 2021. Roots were traced from the trunk of tree species to the exposed root system to confirm the plant species identity of the roots and thus ensure that sampled roots were attached to the tree species being sampled [35–37]. The 60 root samples were taken from *Q. rubra* (3 stands × 10 samples) and *Carya* (3 stands × 10 samples) at the depth of 10 cm and stored at −10 °C until further processing.

The roots present in the samples were carefully separated, washed under running water, placed in a Petri dish filled with distilled water, then examined under a stereomicroscope (Stemi 2000-C, Carl Zeiss, Jena, Germany) at 10–50-fold magnification. The ECM root tips were categorized as either living or dead based on their turgor. The living ECM root tips were classified into morphotypes based on their morphological characteristics (ramification, color, shape, texture, thickness of the mantle, presence of rhizomorphs, and extramatrical hyphae and cystidia) according to previous studies and references therein [15]. If necessary, details of mantle structure and emanating hyphae were examined using a compound microscope (Axio Imager.A1; Carl Zeiss, Jena, Germany) at 400–1000-fold magnification. The number of living ECM roots was counted separately for each morphotype and root sample. The relative abundance of the ECM morphotype was calculated by dividing the

number of ECM root tips of the given morphotype by the total number of living root tips. One to five root tips of each ECM morphotype for each root sample were stored at $-20\text{ }^{\circ}\text{C}$ for molecular analysis.

The DNA was extracted from the individual ECM root tips using the GeneMATRIX Plant and Fungi DNA Purification Kit (EURx, Gdańsk, Poland), following the EURx protocol. The fungal internal transcribed spacer (ITS) region of rDNA was amplified with the Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), using the universal ITS4 primer and the fungal-specific ITS1-F primer, following previous studies [15,38–40]. The amplified ITS region was sequenced at the Laboratory of Molecular Biology of Adam Mickiewicz University using a CEQ 20000XL automatic sequencer with the ITS4 primer. The obtained sequences were revised using BioEdit version 7.2.5 (software available online <https://bioedit.software.informer.com/7.2/> (accessed on 30 March 2022)), and compared with the reference ITS sequences from UNITE (<https://unite.ut.ee/> (accessed on 30 March 2022)) and GenBank (<http://ncbi.nlm.nih.gov/genbank/> (accessed on 30 March 2022)) databases. The identified ECM fungi were assigned to phylogenetic lineages as proposed by Tedersoo et al. [41]. The frequency of ECM phylogenetic lineages was calculated as a percentage of root samples where the lineage was identified in relation to all root samples for *Q. rubra* or *Carya* trees separately. The non-ectomycorrhizal fungi were identified based on UNITE and the literature [41,42].

The diversity and composition of the ECM assemblages were analyzed using the Shannon–Wiener (H') index, dominance index (D), and mean species richness calculated in PAST version 2.17. The similarity and taxa composition of ECM assemblages were analyzed using one-way analysis of similarities (ANOSIM) and non-metric multidimensional scaling (NMDS) with the Bray–Curtis method in PAST version 2.17. Functional diversity of ECM fungi was assessed as the share of exploration types of ectomycorrhizas, identified according to the classification proposed by Agerer [43] and used in further studies [42,44–47].

3. Results

The morphological assessment conducted on 36,023 ECM root tips revealed 19,420 living ECM root tips. A higher share of living ECM roots was noted on *Carya* than *Q. rubra*. More dead ECM roots and a lower total number of roots (both living and dead) were on *Q. rubra* roots (Table 2). Out of living root tips, 290 putative morphotypes were separated, and based on the results of the molecular analysis, 73 ECM fungal taxa were detected. No alien ECM fungi were detected. Non-ectomycorrhizal taxa such as *Mycena*, *Mortierella*, and *Trichoderma* were excluded from the analyses. The ITS sequences for identified ECM taxa were deposited in GenBank (accession numbers ON129102–ON129172), separately for the ECM symbionts on the roots of *Q. rubra* and *Carya* trees.

We identified 23 phylogenetic lineages of ECM fungi, which contained 28 genera. Phylogenetic lineages /*tomentella*-*thelephora* and /*russula*-*lactarius* were represented by the highest number of taxa (Table 3). Additionally, 50% of ECM root tips in all root samples were formed by the five most abundant lineages of Basidiomycota (/ *tomentella*-*thelephora*, /*russula*-*lactarius*, /*pisolithus*-*scleroderma*, /*cortinarius*, and /*entoloma*). The next 33% of all ECM root tips were formed by the four most abundant lineages of Ascomycota (/ *genea*-*humaria*, /*tuber*-*helvella*, /*cenococcum*, and /*galactinia*). The rest 14 lineages taken together constituted 17% of all ECM root tips (Figure 2).

Table 2. Quantitative parameters of tested ectomycorrhizal roots and qualitative data of ECM fungal assemblages on *Carya* and *Q. rubra* roots (ECM taxa richness, diversity indices).

Site	Location 1		Location 2		Loc. 3	Loc. 4	Total	
Stand Type	<i>Carya lacinosa</i>	<i>Quercus rubra</i>	<i>Carya ovata</i>	<i>Quercus rubra</i>	<i>Carya ovata</i>	<i>Quercus rubra</i>	<i>Carya</i>	<i>Q. rubra</i>
Tested ECM roots	9517	5453	4665	4175	7288	5132	21,470	14,760
Living ECM roots (%)	50.5 ± 18.8 ab *	38.1 ± 21.6 -	71.9 ± 6.3 a	46.5 ± 22.9 -	69 ± 9.1 b	40.5 ± 20.6 -	63.8 ± 15.5 A *	41.7 ± 21.3 B
Dead ECM roots (%)	49.5 ± 18.8 a	62 ± 21.6 -	27.8 ± 6.3 b	53.5 ± 22.9 -	31 ± 9.1 ab	59.5 ± 20.6 -	36.2 ± 15.5 -	58.3 ± 21.3 -
ECM taxa richness	14	13	14	17	19	23	40	44
mean taxa richness per tree *	5.9 ± 1.79 a	4.2 ± 1.03 -	3.6 ± 1.07 b	4.4 ± 0.97 -	5.6 ± 1.51 a	4.1 ± 1.20 -	5.03 ± 1.77 A	4.07 ± 1.05 B
Dominance index (D)	0.29 ± 0.21	0.42 ± 0.16	0.45 ± 0.14	0.35 ± 0.17	0.29 ± 0.09	0.38 ± 0.1	0.34 ± 0.17	0.38 ± 0.14
Shannon index (H')	1.50 ± 0.49 a	1.09 ± 0.32 -	0.98 ± 0.29 b	1.26 ± 0.36 -	1.43 ± 0.28 a	1.14 ± 0.25 -	1.30 ± 0.42 -	1.16 ± 0.31 -

* different lower cases (a, b) indicate significant differences between *Q. rubra* and *Carya* stands; capital letters (A, B) indicate significant differences between *Q. rubra* and *Carya* trees ($p < 0.05$, Tukey's test).

Table 3. The phylogenetic lineages of ectomycorrhizal fungi represented by identified ECM fungal taxa.

Order	Phylogenetic Lineage	Fungal Taxon	Score	E-Value	Prct	Reference	
						Sequence	SH Code
Thelephorales	/tomentella-thelephora	<i>Tomentella bryophila</i>	1138	0.0	100.00	UDB000035	SH1528411.08FU
		<i>Tomentella galzinii</i>	998	0.0	99.82	UDB003321	SH1502262.08FU
		<i>Tomentella radiosia</i>	1070	0.0	100.00	UDB017828	SH1502188.08FU
		<i>Tomentella</i> sp. 1	972	0.0	99.08	UDB020340	SH1528408.08FU
		<i>Tomentella</i> sp. 2	1131	0.0	99.68	EU668215	SH1503224.08FU
		<i>Tomentella</i> sp. 3	1086	0.0	98.54	KC965245	SH1503353.08FU
		<i>Tomentella</i> sp. 4	1085	0.0	100.00	KX438351	SH1502482.08FU
		<i>Tomentella</i> sp. 5	662	0.0	100.00	JQ982968	SH1528467.08FU
		<i>Tomentella</i> sp. 6	643	0.0	99.72	MG835439	SH1503546.08FU
		<i>Tomentella</i> sp. 7	193	2.95×10^{-48}	100.00	JQ31864	SH1528406.08FU
		<i>Tomentella</i> sp. 8	1079	0.0	99.66	JX316580	SH1502536.08FU
		<i>Tomentella</i> sp. 9	765	0.0	96.96	MN947374	SH1502214.08FU
		<i>Thelephora terrestris</i>	1151	0.0	100.00	MT644883	SH1502189.08FU
Russulales	/russula-lactarius	<i>Russula amoenolens</i>	1101	0.0	100.00	MW036173	SH1569721.08FU
		<i>Russula fellea</i>	1116	0.0	100.00	UDB000314	SH1509028.08FU
		<i>Russula graveolens</i>	715	0.0	100.00	UDB002538	SH1538879.08FU
		<i>Russula nobilis</i>	1085	0.0	100.00	UDB000112	SH1509039.08FU
		<i>Russula recondita</i>	1147	0.0	100.00	KJ530756	SH1528312.08FU
		<i>Russula velenovskyi</i>	1064	0.0	100.00	UDB017880	SH1538848.08FU
		<i>Russula violacea</i>	649	0.0	100.00	MT005921	SH1538991.08FU
		<i>Lactarius decipiens</i>	1035	0.0	100.00	UDB038441	SH1632888.08FU
	/paxillus-gyrodon	<i>Melanogaster ambiguus</i>	1251	0.0	99.56	KX438335	SH1540279.08FU
Boletales	/boletus	<i>Xerocomellus chrysenteron</i>	1166	0.0	99.84	UDB024777	SH1633178.08FU
		<i>Xerocomellus cisalpinus</i>	1199	0.0	100.00	HM190074	SH1508912.08FU
		<i>Xerocomellus porosporus</i>	830	0.0	99.56	UDB001226	SH1508917.08FU
		<i>Imleria badia</i>	870	0.0	100.00	MT278249	SH1511625.08FU
		/pisolithus-scleroderma	<i>Scleroderma areolatum</i>	1269	0.0	100.00	UDB031438

Table 3. Cont.

Order	Phylogenetic Lineage	Fungal Taxon	Score	E-Value	Prct	Reference		
						Sequence	SH Code	
Atheliales	/piloderma	<i>Piloderma lanatum</i>	662	0.0	99.72	UDB001744	SH1544816.08FU	
		<i>Piloderma</i> sp.	658	0.0	100.00	AM161525	SH1544827.08FU	
Sebacinales	/sebacina	<i>Helvellosebacina</i> sp.	776	0.0	99.53	UDB016423	SH1515900.08FU	
Agaricales	/entoloma	<i>Entoloma bryorum</i>	941	0.0	100.00	LN850539	SH1605002.08FU	
		<i>Entoloma</i> sp.	505	1.56×10^{-141}	100.00	LN850539	SH1605002.08FU	
		<i>Entoloma griseorugulosum</i>	1219	0.0	100.00	LN850589	SH1605033.08FU	
	/hebeloma- alnicola	<i>Hebeloma erebium</i>	1101	0.0	100.00	KT218373	SH1563789.08FU	
		<i>Hymenogaster arenarius</i>	1062	0.0	100.00	MT005976	SH1563796.08FU	
	/inocybe	<i>Inocybe asterospora</i>	1142	0.0	100.00	UDB000098	SH1645682.08FU	
		<i>Inocybe pusio</i>	1146	0.0	99.68	UDB031390	SH1527090.08FU	
		<i>Inocybe splendens</i>	1066	0.0	100.00	MN947377	SH1527479.08FU	
		<i>Inocybe umbrinella</i>	765	0.0	99.06	FJ904166	SH1649739.08FU	
	/laccaria	<i>Laccaria amethystina</i>	998	0.0	100.00	UDB000039	SH1553002.08FU	
		<i>Laccaria laccata</i>	1086	0.0	99.66	KM067834	SH1553002.08FU	
	/cortinarius	<i>Cortinarius hirtus</i>	678	0.0	100.00	MT935141	SH1545222.08FU	
<i>Cortinarius incisus</i>		1153	0.0	99.53	HQ204635	SH1504210.08FU		
<i>Cortinarius torvus</i>		955	0.0	100.00	UDB000091	SH1545291.08FU		
/genea-humaria	<i>Genea hispidula</i>	944	0.0	100.00	UDB001408	SH1539219.08FU		
	<i>Humaria</i> sp. 1	1112	0.0	100.00	MH834482	SH1608584.08FU		
	<i>Humaria</i> sp. 2	1112	0.0	100.00	MG019765	SH1608585.08FU		
	<i>Tuber maculatum</i>	815	0.0	99.76	UDB027452	SH1648382.08FU		
	<i>Tuber puberulum</i>	865	0.0	100.00	MT005934	SH2732293.08FU		
/tuber-helvella	<i>Tuber rufum</i>	1059	0.0	99.83	UDB033109	SH1544218.08FU		
	<i>Helvella</i> sp.	846	0.0	100.00	UDB019802	SH1539841.08FU		
	<i>Peziza michelii</i>	861	0.0	100.00	UDB0778643	SH1515878.08FU		
Pezizales	/galactinia	<i>Peziza</i> sp.	942	0.0	99.81	MG835428	SH1643050.08FU	
		<i>Peziza ostracoderma</i>	966	0.0	99.25	UDB0778581	SH1630799.08FU	
	/terfezia-peziza depressa	<i>Terfezia</i> sp.	966	0.0	99.25	UDB0778581	SH1630799.08FU	
	/hydnotrya	<i>Hydnotrya tulasnei</i>	1142	0.0	100.00	HM189757	SH1572661.08FU	
	/marcelleina- peziza gerardii	<i>Hydnobolites</i> sp.	787	0.0	99.77	MG835420	SH1539144.08FU	
	/pachyphloeus- amylascus	<i>Pachyphloides</i> sp.	926	0.0	99.22	UDB0323731	SH2723524.08FU	
	/tarzetta	<i>Tarzetta scotica</i>	813	0.0	98.48	JF927143	SH1647422.08FU	
	Eurotiales	/elaphomyces	<i>Elaphomyces muricatus</i>	1022	0.0	100.00	JQ272414	SH1587495.08FU
	Helotiales	/meliniomyces	<i>Hyaloscypha bicolor</i>	1149	0.0	99.84	HM164658	SH1523753.08FU
	Mytilinidiales	/cenococcum	<i>Cenococcum sensu lato</i>	n/a *	-	-	-	-

Table 3. Cont.

Order	Phylogenetic Lineage	Fungal Taxon	Score	E-Value	Prct	Reference	
						Sequence	SH Code
-	-	UN Thelephoraceae 1	n/a	-	-	-	-
		UN Thelephoraceae 2	n/a	-	-	-	-
		UN Thelephoraceae 3	n/a	-	-	-	-
		UN Thelephoraceae 4	n/a	-	-	-	-
-	-	UN Russulaceae 1	n/a	-	-	-	-
		UN Russulaceae 2	n/a	-	-	-	-
-	-	UN Cortinariaceae 1	n/a	-	-	-	-
		UN Cortinariaceae 2	n/a	-	-	-	-
		UN Cortinariaceae 3	n/a	-	-	-	-
		UN Cortinariaceae 4	n/a	-	-	-	-
-	-	UN Boletaceae 1	n/a	-	-	-	-
		UN Boletaceae 2	n/a	-	-	-	-

* not applicable—failed to amplify or sequence, and due to the low abundance (several ECM root tips per morphotype), unable to reanalyze; identified based on the morphology of ECM tips and our other studies of ECM root tips.

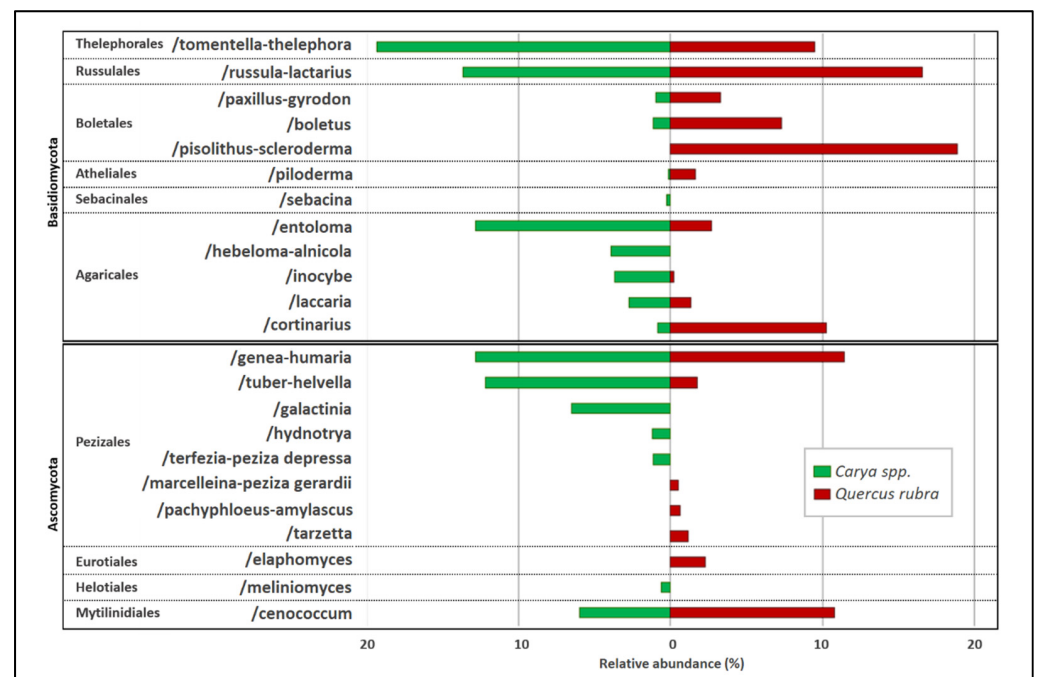


Figure 2. Relative abundance of ECM phylogenetic lineages of fungi on *Carya* and *Q. rubra* roots.

Out of 23 ECM phylogenetic lineages, 12 lineages were common for both *Q. rubra* and *Carya*, 5 were found only on *Q. rubra*, and 6 lineages were found only on *Carya* roots (Figure 2). Lineages /russula-lactarius, /tomentella-thelephora, /genea-humaria, and /cenococcum were represented by a similar abundance of ECM tips on *Q. rubra* and *Carya* roots. The largest difference was observed among lineages from Boletales (/pisolithus-scleroderma; /boletus; and /paxillus-gyrodon), which were represented by 29.5% of ECM tips on *Q. rubra* roots and 2.1% of ECM tips on *Carya* roots (Figure 2). The long-distance exploration type (formed by Boletales fungi) and medium-distance exploration type of ectomycorrhizae were most abundant on *Q. rubra* (52.9% of ECM roots). On the contrary,

80.1% of ECM tips on *Carya* roots were formed by ECM fungi of contact and short-distance exploration types of ectomycorrhizae (Figure 3).

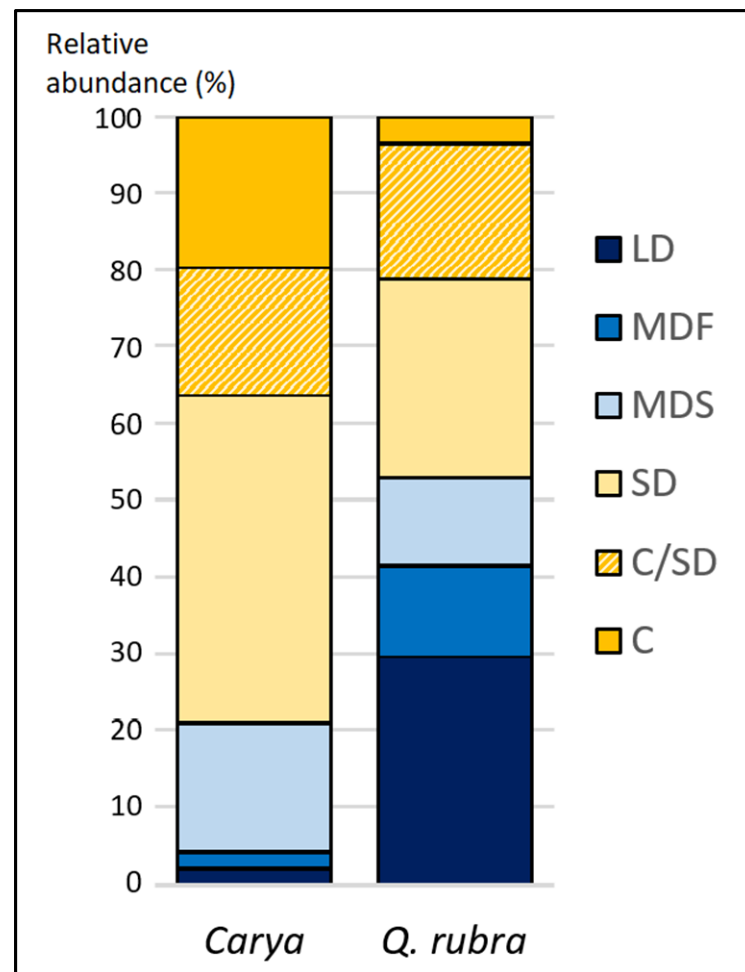


Figure 3. Relative abundance of exploration types of ectomycorrhizas on *Carya* and *Q. rubra* roots based on the classification proposed by Agerer [43]: C—contact, SD—short-distance, C/SD—contact or short distance; MDS—medium-distance smooth, MDF—medium-distance fringe, and LD—long-distance exploration type.

An ANOSIM revealed that differences in ECM fungal assemblages among *Carya* and *Q. rubra* trees were significant but not high (ANOSIM $R = 0.21$, $p = 0.0001$). Non-metric multidimensional scaling ordination of ECM fungal assemblages of *Carya* and *Q. rubra* partly overlapped each other (Figure 4). The values of the Shannon–Wiener (H') diversity index and Simpson’s dominance index (D) were similar for *Carya* and *Q. rubra* stands (Table 2). Significant differences were noted for the number of living ECM roots and mean taxa richness; both parameters had higher values on *Carya* than *Q. rubra* roots (Table 2).

The highest frequency was noted for phylogenetic lineages /tomentella-thelephora, /russula-lactarius, /genea-humaria, and /cenococcum (Table S1), which was concurrent with the high abundance of these lineages (Figure 2). Medium to high frequency (25–40%) was noted for lineages /entoloma, /tuber-helvella, /inocybe, and /hebeloma-alnicola on *Carya*, and for lineages /cortinarius and /pisolithus-scleroderma on *Q. rubra* roots. About half of the lineages on *Carya* (44.4%) and *Q. rubra* (52.9%) were represented by low frequency up to 10% (Table S1).

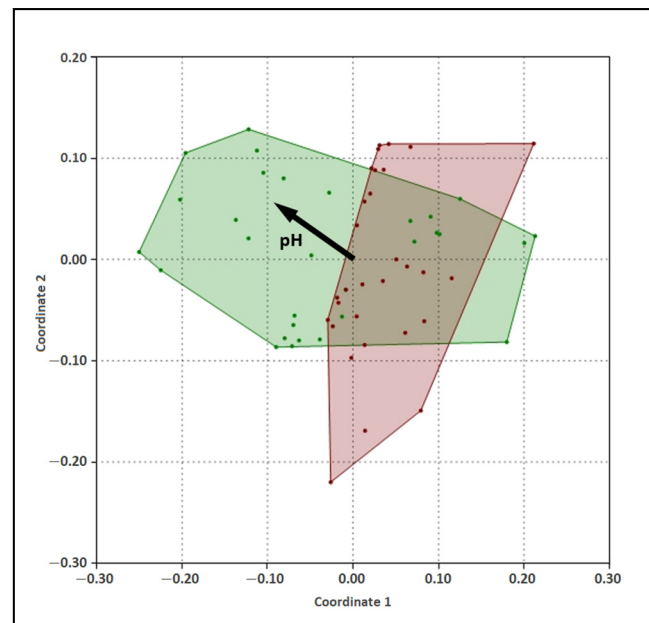


Figure 4. Non-metric multidimensional scaling (NMDS) plots based on the Jaccard similarity index of ECM fungal symbionts associated with *Carya* (green) and *Q. rubra* (red).

4. Discussion

We presented the first comparative study of ECM fungal assemblages on the roots of invasive *Q. rubra* and non-invasive *Carya* species under common garden conditions in Central Europe. We showed that the share of living ECM roots was significantly lower on *Q. rubra* than *Carya* roots. We observed less living and more dead ECM roots on *Q. rubra* than *Carya* roots, regardless of the surroundings (Table 2). It is likely that the difference in the number of living ECM roots was driven by species-specific differences between *Q. rubra* and *Carya*. *Quercus rubra* can modify soil's physical and chemical properties and cause an accumulation of tannins in the organic soil horizon relative to native plants [21], but no similar effect was observed for *Carya* trees [15,22]. Tannins enter the soil as a component of litter, slowing down decomposition and microbial activities [48–50], which can affect a higher share of dead ECM roots on *Q. rubra* than *Carya* (Table 2).

Some authors pointed out that highly invasive tree species are more dependent on ECM symbiosis [51], although plant species characterized by obligate ECM associations are rarely naturalized outside the native range (45% of introduced species) compared to plants of facultative mycorrhizal symbiosis (76% of introduced species) [52]. The aforementioned studies of highly invasive trees were conducted on the *Pinus* species only, regardless of the environmental requirements of single pine species [51]. Pine species differ in soil preferences, climatic niches, and general environmental requirements; thus, the area of their native range varies greatly from one pine species to another [53]. The analysis of hundreds of alien tree species on a global scale showed that alien tree species, which form various types of symbiosis with soil microbial mutualists, represent about 30% of all invasive tree species and about 70% of invasive tree species spread globally [5]. It indicates that a lower dependency on one type of symbiosis is one of the crucial competitive advantages of invasive tree species over other native and non-native tree species. *Quercus* section *Lobatae* (among others *Q. rubra*) can enter different types of mycorrhizal symbiosis in their native range in North America [27,28,54], unlike white oaks (*Quercus* sect. *Leucobalanus*), which are neither native to North America [54] nor native to Europe [28]. European *Quercus* sect. *Leucobalanus* (e.g., *Q. robur* and *Q. petraea*) are characterized by high ECM colonization up to 100% [55], similar to high ECM colonization on *Carya* inside the native oak forest or native trees therein [15]. Here, we showed that in the case of invasive *Q. rubra*, a considerably

lower share of roots was covered with living ECM fungi than for non-invasive *Carya* trees nearby (Table 2).

4.1. Ectomycorrhizal Taxa Richness

The total richness of ECM symbionts was comparable for *Q. rubra* and *Carya*, although mean taxa richness was slightly lower on *Q. rubra* (Table 2). Climate, soil conditions, and set of neighboring trees linked to the geographical location of the study site strongly influence fungal species richness and composition of ECM assemblages for both native and alien tree species, including the tested *Q. rubra* and *Carya* [15,33,34,38,39]. Therefore, the comparison of our results with other studies of ECM roots on tested species, conducted under different climatic and soil conditions, is limited. However, in previous studies of ECM assemblages of tested trees in Europe, higher ECM species richness was noted on *Carya* [14,15] than *Q. rubra* roots [33,34]. Nevertheless, total ECM taxa richness (but not ECM composition) was similar and low for both invasive *Q. rubra* and native *Q. robur* tested under common garden conditions [33], showing the influence of the set of environmental factors linked to the geographical location. The presence and abundance of ECM fungi are shaped by both tree-specific factors such as tree age, the taxonomic position of tree species, and the affinity of trees for ECM fungi [38,39,44] and aforementioned site-specific factors [15,38,39,56,57]. Under forest conditions, numerous factors such as small-scale disturbances or competition are unlikely to identify backward in time. We used well-documented collections of alien trees grown together in a dendrological garden for over 150 years, which allowed us to omit a part of the abovementioned random variability.

4.2. Composition of Ectomycorrhizal Assemblages

Under common garden conditions, we found significant differences in taxonomic and functional diversity among ECM assemblages of *Q. rubra* and *Carya* (Figures 2–4), although the dominance (D) and Shannon (H') diversity indices were similar (Table 2). Different composition but similar ECM taxa richness was noted for alien tree species compared with native trees nearby [15,33,58,59].

The composition of ECM fungi appeared to be more susceptible to changes than the species richness. The hypothesis of functional redundancy of ECM communities assumes that trees uphold the symbiosis with a much higher number of ECM fungi than are necessary for their functioning at a specific point in time and place [60,61]. This mechanism seems to be an evolutionary adaptation of trees, long-lived organisms, to the fluctuations of environmental conditions. As a result, the share of ECM roots belonging to different taxonomic and functional groups of ECM fungi is shifting along time and environmental gradients, but the total number of ECM taxa is comparable [60–63]. Functional redundancy explains the common pattern of ECM fungal assemblages when few dominant taxa form the majority of ECM root tips (over 70%) but a majority of ECM fungal taxa (up to 95%) is represented by a low abundance of ECM roots (about <1–3% of all ECM roots per taxa). Moreover, it also explains the surprising identity of certain ECM fungal taxa, such as a few ECM roots of fungi closely associated with young trees on the roots of mature trees [44], or conversely, fungal species associated with later stages of the forest which occurs on seedlings in the forest nurseries [39].

On the other hand, some ECM genera contain dozens and hundreds of closely related species (e.g., *Russula*, *Tomentella*, *Cortinarius*, and *Entoloma*), which can play a similar function in the ecosystem, while other genera are represented by a limited pool of species (e.g., *Humaria*, *Genea*, *Piloderma*, *Paxillus*, and *Cenococcum*); thus, they may never have a species-rich representation in any ECM assemblages [41,42]. Although some closely related ECM fungal taxa (e.g., *Cortinarius* or *Russula* species) can also have varied ecological functions and requirements (e.g., pine-associated and oak-associated *Cortinarius* species), for individual ECM fungal species, they are poorly understood. Otherwise, numerous species of mentioned ECM fungal genera co-exist in similar native forest ecosystems and most likely have comparable requirements.

In view of the above reasons, we focused on a higher level of taxonomic diversity represented by phylogenetic lineages, which merge close-related ECM taxa [41,42], and on the functional diversity of ECM assemblages (exploration types of ectomycorrhizas). We identified only ECM fungal taxa native to Europe, previously reported as symbionts of native tree species. Identified ECM taxa belonged to the phylogenetic lineages, which were found in the studies of ECM symbionts of *Q. rubra* and *Carya* in their native range in North America [16,64,65] and previous studies on these trees in Europe [14,15,33]. We found no ECM fungus alien to Europe, exactly as in previous studies of *Q. rubra* and *Carya* in Europe [14,15,33,34], probably because of the fact that tested trees were imported to Europe as seeds but alien ECM fungi are usually introduced with soil or seedlings [32,66], which would explain the lack of alien ECM fungal species on the roots of tested trees.

4.3. Functional Diversity—Phylogenetic Lineages and Exploration Types of Ectomycorrhizas

The ANOSIM analyses and NMDS ordination showed a significant difference between tested ECM assemblages of *Q. rubra* and *Carya* trees under common garden conditions (Figure 4). The partly overlapped shape of the NMDS ordination is similar to the ordination for *Carya* monocultures vs. native trees in the surroundings [15]. In both mentioned cases, the overlapped part of the ordination is driven by the group of ubiquitous ECM symbionts, which enters ECM symbiosis with numerous Fagales genera (among others, *Quercus* and *Carya*) and even with distantly related Pinales (e.g., *Pinus*) [23].

We revealed a significant difference in the share of long-distance (LD) exploration type of ectomycorrhizas on *Q. rubra* (30% of all ECM roots) in comparison to *Carya* roots (2%; Figure 3). The long-distance exploration type, characterized by the highest mycelial absorbing surface area among exploration types of ectomycorrhizas, is formed exclusively by Boletales [42,43,46,47], including all ECM genera of Boletales, except *Chroogomphus* and *Gomphidius* [42]. *Pisolithus* and *Scleroderma* (lineage /pisolithus-scleroderma, LD) on the roots of invasive *Eucalyptus globulus* revealed a higher growth increase at least in a homogenized sterile mineral soil than other ECM species, such as *Hydnangium* (/laccaria), with medium-distance exploration type [67]. Fungi of long-distance exploration type are global drivers of pine invasion (lineage /suillus-rhizopogon; [66]) and are even noted as rapidly spreading invasive species on their own (lineage /boletus), entering the ECM symbiosis with native trees [68], what's an unusual phenomenon for alien ECM fungi in Europe [32].

We previously noted a curious dissimilarity between the LD exploration type on *Carya* roots (0–5%) compared to the surrounding native forest (up to 30–40%), which was driven by the abundance of ECM roots of *Paxillus involutus* (/paxillus-gyrodon, LD) [15]. *Paxillus involutus* is well-known as a ubiquitous species, which forms ECM symbiosis with a wide range of tree genera [69] but not *Carya* trees; therefore, no affinity between *Carya* and *Paxillus* has been suggested [15]. It should be noted that the diversity of Boletales in subtropical and tropical regions is notably higher than in Europe. For example, the genus *Tylopilus* contains over 100 ECM fungal species; about 40 are known as native to North America, but only one, *T. fellus*, is native to Europe. An opposite case has not been found. At the higher taxonomic level, a few times more ECM genera of Boletales are known to be native to America, Australia, and Asia than Europe [70].

Notably, ECM fungi of Boletales, such as *Astraeus*, *Pisolithus*, *Boletus*, and *Xerocomus*, were identified on *Carya* in North America [64,65] but not in Europe [14,15]. *Astraeus* and *Pisolithus* are rare in Central and Northern Europe, but *Boletus* and *Xerocomus* are common among ECM symbionts of European trees, and their sporocarps were frequently found in the surroundings of the study stands (personal observations). Sporocarps of *Boletus reticulatus* were even noted under a canopy of native *Carpinus betulus* (Betulaceae, Fagales) during the sampling (Locations 1 and 4). Numerous genera of Boletales were found in native forests in the surrounding *Carya* stands during the previous studies (e.g., *Boletus*, *Tylopilus*, *Leccinum*, *Xerocomus*, *Gyroporus*, and *Paxillus* species), but none of them were found on *Carya* roots [15], which suggests the low level of alignment between native

European boletes and North American trees. Co-invasion between North American trees and alien ECM fungi were noted, among others, for alien-to-Europe genus *Pseudostuga* and its ECM symbiont *Suillus lakei* (Boletales), and crucially, the presence of *Suillus lakei* enables the global invasion of *Pseudostuga* [71]. In this study, we noted numerous ectomycorrhizas of native *Scleroderma* species on *Q. rubra* (Figure 2), unlike *Carya* roots. The lack of appropriate ECM fungi of Boletales does not seem to be a limitation for invasive *Q. rubra*.

We have shown a similar abundance of ECM phylogenetic lineage /russula-lactarius, but composed almost entirely of *Russula* species. *Russula* species, in contrast to *Lactarius* species, were more often found on *Carya* roots in previous studies [14,15]. The lack of symbiotic affinity was found for *Carya* and native *Lactarius* species [15]. A comparison of ECM assemblages of invasive *Q. rubra* and native *Q. robur* has shown a considerable difference in the abundance of ECM roots formed by *L. quietus*, typical ECM symbiont of European oaks, on the roots of native *Q. robur* (33% of all ECM roots) compared to no ECM roots formed by *L. quietus* on *Q. rubra*. This notable difference among trees grown side-by-side reinforces our previous hypothesis of a lack of affinity (or limited affinity) between North American trees (*Carya*, *Quercus* sect. *Lobatae*) and a part of native ECM fungi [15].

Previously, we hypothesized that *Genea* and *Humaria* (lineage /genea-humaria) may functionally substitute lineage /tuber-helvella [15], which dominates the ECM assemblages of *Carya* on more alkaline soils in North America [64]. In this study, we showed no similar relations between the abundance of /genea-humaria and /tuber-helvella. Moreover, we noted a similar abundance of /genea-humaria on *Q. rubra* and *Carya* roots, but a considerably higher abundance of *Tuber* (lineage /tuber-helvella) on *Carya* (about 12%) than *Q. rubra* (about 2%). Here, we recorded a similar proportion of lineages /tuber-helvella, /galactinia, /russula-lactarius, and /tomentella-thelephora, except lineage /pisolithus-scleroderma (Boletales), on the roots of tested *Carya* trees compared to *Carya* in native forests in North America [64]. Under similar soil conditions, ECM roots of *Tuber* (lineage /tuber-helvella) were present but sparse on *Q. rubra* roots. *Carya* is an appropriate partner for numerous *Tuber* species, and thus are commonly used in truffles orchards in North America [64,65], and even successfully inoculated with valuable European truffles [72,73]. It would explain the observed differences in the abundance of lineage /tuber-helvella but not /genea-humaria, which form the same short-distance exploration type of ectomycorrhizas as *Tuber*. Moreover, native trees in the surroundings, e.g., *Tilia cordata*, *Carpinus betulus*, *Fagus sylvatica*, and *Q. robur*, are tree partners of numerous native truffles [39,55,74–76], as well as commercial species used in truffle orchards [74,77].

In summary, we described for the first time the ECM assemblages of *Q. rubra* and *Carya* trees grown under common garden conditions outside their native range. We found that this alien tree species upholds a similar richness but different composition of ECM fungi. The ability to accept local ECM fungal symbionts is the crucial factor in the successful acclimatization of alien tree species outside their native range [78]; however, the differences in ECM strategy between invasive and non-invasive alien tree species is poorly recognized. Some older studies of ECM fungi associated with invasive *Q. rubra* provide ambiguous results, incompatible with the later studies, apparently due to the technical errors in older methods of root sampling. Therefore, we traced the sampled roots of trees to the trunk of the tree carefully and precisely. All roots of unclear origin were eliminated. Finally, we noted a considerably high abundance of long-distance exploration type formed by ECM fungi of Boletales order on the roots of invasive *Q. rubra*, unlike non-invasive *Carya* trees, but a high abundance of economically important fungus *Tuber* on *Carya* roots. To specify the mutual compatibility between alien tree species and native bolete species such as *Paxillus* or *Boletus*, further studies under controlled conditions are recommended. Moreover, we suggest the comparative tests of European trees with boletes native to North America and North American trees with bolete species native to Europe.

Supplementary Materials: The following are available online at <https://www.mdpi.com/xxx/s1>, Table S1: The frequency of ECM phylogenetic lineages on the roots of tested trees.

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