



Root anatomical adaptations of contrasting ectomycorrhizal exploration types in *Pinus sylvestris* and *Quercus petraea* across soil horizons

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Abstract

Aims The anatomical characteristics of ectomycorrhizal exploration types in response to soil variability remain insufficiently understood. We examined the root anatomy of contact and long-distance exploration types in *Pinus sylvestris* and *Quercus petraea*, species with distinct ecological needs, across different soil horizons.

Methods The diameter of ectomycorrhizal roots, the root absorptive traits i.e. proportion of cortex and mantle area, the percentage stele in the diameter, and the weighted average diameter of vessels (Ra) in the ectomycorrhizas were measured within ectomycorrhizas collected from organic and mineral soils across the soil profile.

Results The absorptive traits varied along soil horizons, in which water and nutrient availability changed

inversely. The proportion of cortex was associated with exploration type, but was not specific to tree species. However, the ectomycorrhizal diameter and the percentage of mantle within the root forming contact exploration type of *P. sylvestris* showed no variation among soil horizons. In contrast, the soil horizon significantly influenced all root anatomical traits in the contact exploration type of *Q. petraea* by enhancing the contribution of the absorption area of the root area, mainly in the illuvial horizon, but reaching the smallest value in the organic horizon. The Ra and the cell wall thickness of the vessels were strongly dependent on tree species. With increasing soil depth, Ra in *Q. petraea* increased, and stele proportion in root diameter decreased.

Conclusion The results suggest that water acquisition traits differ among tree species, but traits associated with nutrient absorption (proportion of cortex and mantle area) within specific soil horizons are closely related to the ectomycorrhizal exploration type.

Keywords Nutrient availability · Exploration type · Cortex · Hydraulic traits · Scots pine · Sessile oak

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Introduction

Fine roots are responsible for the uptake of water and nutrients in shallow soil layers, but resource acquisition strategies from deeper layers are much less studied and understood (Maeght et al. 2013; Rewald et al.

2014). Even less is known about their large functional variability to adjust uptake of water and nutrients within conditions of the specific soil profile (Wang et al. 2023). Water availability is higher in deeper profiles where the availability of nutrients is relatively low, thus trees may adjust their fine root physiology and anatomy to expand soil exploitation, either enhancing hydraulic efficiency or nutrient acquisition, respectively (McElrone et al. 2007).

Soil edaphic conditions can significantly influence the functional capacity of roots to absorb water and nutrients (Freschet et al. 2017). However, it remains unclear how plants adjust to growth in different soil layers to optimize these functions. For instance deep roots often have thicker diameters (Pregitzer et al. 1998; Wang et al. 2016), lower fungal colonization (Neville et al. 2002; Sun et al. 2010), lower nitrogen concentration (Neville et al. 2002; Sun et al. 2010), and lower respiration rates (Pregitzer et al. 1998; Burton et al. 2012). These changes are of particular importance for absorptive fine root tips responsible for water acquisition (McCormack et al. 2015). In *Juglans mandshurica* and *Fraxinus mandshurica*, root diameter increases with soil depth, as shown by Wang et al. (2016), while *Phellodendron amurense* exhibits the opposite trend. However, it remains an open question whether larger diameter is a result of larger stele or thicker parenchyma cortex. As thicker roots should be able to conduct more water in deep layers, plants exhibit production of roots with larger steles that due numerous and larger conduits (e.g., vessels or tracheas) showing higher transport capacity to drain abundant water in deep layers that is very frequently inaccessible in shallow layers. Supporting this view, studies show that the deep roots of four tree species had higher hydraulic conductivity than shallow roots due to increased conduit diameters (McElrone et al. 2004). On the other hand, the enhancement of parenchyma cortex serving to uptake resources in absorptive roots can enhance uptake potential of absorptive roots (McCormack et al. 2015; Guo et al. 2008), but consequently enhances the hydraulic resistance of water transport from the root surface to the conduits located in the stele (Steudle and Peterson 1998; Huang and Eissenstat 2000). Thus having a large cortex is likely rather to benefit for roots located in shallow soil layers not only because this may be a strategy to accelerate acquisition of nutrients, primarily concentrated near the soil surface (Schenk

2008; Ugawa et al. 2010). Additionally, a larger cortex provides more space for mycorrhizal colonization, further boosting nutrient uptake via mycotrophy (Brundrett 2002; Comas et al. 2012). Moreover, the structure and thickness of the fungal mantle - responsible for intense nutrient uptake and storage - itself may also represent a functional trade-off depending on fungal species and environmental conditions (Ostonen and Löhmus 2003). However, studies on the relationships between root anatomy, mycorrhizal colonization, and their variability at different soil depths are still limited (Trocha et al. 2017), despite, mycorrhizal symbiosis significantly influences the functionality of different root traits.

Ectomycorrhizal fungi (EcM) exhibit various “soil exploration types” classified by the morphological traits of colonized root tips and the structure of emerging mycelium (Agerer 2001). Fungi with no or minimal cords (including contact, short-, and medium-range smooth types) maximize the absorptive surface area of hydrophilic hyphae that penetrate the soil. This enhances rapid nitrogen uptake and reduces resource leaching (Hobbie and Agerer 2010; Bahr et al. 2015). In contrast, exploration types with hydrophobic mantles and cords, including medium distance fringe and long distance types, may grow more strategically toward specific patches of immobile organic resources (Finlay and Read 1986; Cairney 2005; Hobbie and Agerer 2010). Moreover, trees are believed to have the ability to modify the same type of fungal exploration, enhancing nitrogen uptake from diverse soils (Trocha et al. 2021). Given that EcM ‘contact’ and ‘short-distance’ types promote rapid uptake of mobile N in regions with high N availability, while ‘long-distance’ explorers focus on spatially concentrated and widely dispersed soil resources to enhance the nutrient acquisition, especially under N-limited conditions (Fernandez et al. 2017), we anticipate greater adjustment of contact type mycorrhizas with increasing soil depth. However, this “slow growth strategy” reduces their ability to absorb nutrients at greater soil depths (Göransson et al. 2007; da Silva et al. 2011). Additionally, differences in the hydrophobicity of fungal hyphae could impact water uptake (Lehto and Zwiazek 2011), supporting the hypothesis that the primary role of deep roots may be water uptake and transport rather than nutrient acquisition (Pregitzer et al. 1998; Ugawa et al. 2010; Makita et al. 2011; Burton et al. 2012).

Therefore, it remains to be shown if potential differences in water and nutrient absorption between soil profiles can be linked to anatomical root properties of different mycorrhizal types, especially that there is ongoing debate about fine root functioning (considering the fungal phylogeny and root functional traits) when roots are not collected from arbitrarily divided soil layers and species of different ecological requirements (e.g., Pregitzer et al. 1998; Coleman et al. 2004; Hishi et al. 2006; Burton et al. 2012; Wang et al. 2016). Scots pine (a gymnosperm) (Zarzycki et al. 2002; De Chantal et al. 2003) and sessile oak (an angiosperm) (Bréda et al. 1993; Epron and Dreyer 1993) exhibit distinct ecological requirements. Both species form symbiotic relationships with ectomycorrhizal fungi. The root foraging strategies and the root-mycorrhizal fungal continuum are strongly affected by anatomical differences and structural changes within their roots. Angiosperms, such as oaks, generally exhibit thinner roots with a reduced cortex, smaller vessels, and a larger proportion of stele, traits that reflect their adaptation to more water-limited environments (Comas et al. 2012; Kong et al. 2017). As demonstrated by Chen et al. (2016), tree species with thicker roots, such as tulip poplar and pine, show little or no response to changes in nutrient availability. In contrast, species with thinner roots, like oaks, are more responsive, adjusting through selective root production or increased investment in mycorrhizal dispersal. Coarser-rooted plants face limitations in nutrient foraging and absorption, making them more dependent on medium- and long-distance mycorrhizal explorers, which can access resources beyond the root depletion zone (Chen et al. 2018a, b). Despite this, studies linking root functional traits with EcM associations are relatively rare (Ostonen et al. 2011; Chen et al. 2016, 2018a). Linking root functional traits with EcM associations are rare (Ostonen et al. 2011; Chen et al. 2016, 2018a). The sessile oak (*Quercus petraea* Liebl.) also develops an extensive taproot system, facilitating the uptake of water and nutrients from deep soil layers (Hoffman and Lyr 1967). Therefore, there may be differences in how oak roots adapt to take up nutrients across different soil profile (Ferio et al. 2003).

In our study, we examined the distribution of anatomical traits of ectomycorrhizal root tips across soil horizons in pioneer Scots pine (*Pinus sylvestris* L.) and sessile oak (*Q. petraea* Liebl.), a

medium-successional deep root species that is adaptable to diverse soil conditions (Trocha et al. 2017, 2021). Our goal was to explore whether contact and long-distance exploration ectomycorrhizas exhibit anatomical variation along the vertical soil profile. Specifically, we examined traits such as root diameter, the proportion of absorptive surface to root diameter, the proportion of stele to root diameter, and the weighted average diameter of vessel (Ra). Our previous research indicated that fine roots all generally had high nitrogen content across different soil horizons, even in those soil horizons with lower nitrogen content (Trocha et al. 2017). At the same time, long-distance morphotypes with higher mycelial biomass are more enriched in ^{15}N than those with lower mycelial biomass (Hobbie and Agerer 2010), suggesting the ability to utilize nutrients from a wide range of soil resources (Pena et al. 2017). Based on this, we hypothesized that contact type roots would be characterized by wider mantle to compensate for lower nutrient availability deeper in the soil profile. Conversely, long-distance morphotypes enhance cortex thickness to explore soil resources at greater depth i.e. thicker cortex may enable more efficient nutrient transfer from broader area of soil. Additionally, thicker the average root vessel diameter will be more pronounced in oaks deeper in the soil profile irrespective of contact type, as oaks are used to uptake deep water (Hoffman and Lyr 1967), in contrast to pines that have a branched, shallow root system (Roberts 1976). Our study contributes to the understanding of how root functional traits are distributed along the soil profile.

Materials and methods

Study sites

The two monoculture stands of *Q. petraea* and *P. sylvestris* are in the Zielonka Forest (52°33' N, 17°06' E), west-central Poland. They are under the management of the Zielonka Experimental Forest District, which belongs to Poznań University of Life Sciences. The two stands are the same age: they were 98–99 years old at the time of root collection (2012–2014). The herb layer consists mainly of *Vaccinium myrtillus* L. and *Dryopteris filix-mas* L. Schott in the *P. sylvestris* stand, and *Calamagrostis arundinacea* L. Roth.

and *D. filix-mas* in the *Q. petraea* stand. Both stands are located close to each other (about 300 m (ca. 984 ft) apart), in sub-compartments 115 c and 115 h. The surface area of sub-compartment 115 c (sessile oak) is 7.32 hm², while the area of sub-compartment 115 h (Scots pine) is 9.11 hm². The two stands were planted according to silviculture standards applied in the Polish forest management system. For *P. sylvestris*, the original number of seedlings planted per one square hectometre is 8–10,000, compared with 6–8,000 for *Q. petraea*. After almost 100 years of forest tending operations, the final number of trees per square hectometre is approximately 400–500 for *P. sylvestris* and 300–400 for *Q. petraea*. The average diameter at breast height was 32 cm in the *P. sylvestris* stand and 30 cm in the *Q. petraea* stand; the average tree height was 28 m and 26 m, respectively. The stocking index for the *P. sylvestris* stand was 1.0; it was 0.9 for the *Q. petraea* stand. In Polish forestry system stocking (index of stocking) is a measure which is determined by the ratio of estimated growing stock of a stand per 1 hm² (merchantable volume) to merchantable growing stock given in tables (for the same tree species, the same site class and the same age – as the sum of merchantable growing stock of the main and secondary forest stand) (Miś and Woźniak 2014; Szymkiewicz 2001).

Climate data

The climate of this part of Poland is transitional between maritime and continental, but it is definitely more strongly influenced by the milder Atlantic climate than the harsh continental one.

The average annual precipitation between 1987 and 2008 was 531 mm/year (range 341.3–654.7 mm/year), spread over a mean of 163 days/year. The mean annual time with snow cover was 46 days. Periods without precipitation usually occur during the warmer part of the year (Grajewski 2011, 2013). The mean annual temperature was 8.7 °C, and the length of the growing season was 200 days. The mean annual number of days without ground frost was 153, and the mean annual sum of insolation was 1378 h. The coldest month was January (mean temperature = −0.7 °C), and the warmest month was July (mean temperature = 19.2 °C). These data were based on measurements from the local meteorological station, about 3 km (ca. 1.8 miles) from the stands.

Root collection

The root collection occurred each mid-September for the three consecutive years (2012–2014) when this project was conducted. We dug up six soil pits each year: three in the *P. sylvestris* and three in the *Q. petraea* stands (hence, nine soil pits in total across three vegetation seasons in each species). Each pit was about 1 × 2 × 2 m (W × L × D) and was dug up in a different place each year. To avoid the so-called “edge effect,” we ensured that the pits were situated near the centers of the two sub-compartments. While choosing pit locations, we also considered microhabitat variability (spatial heterogeneity). Every season, the distance between the pits in each stand was between 50 and 100 m.

After the soil profile (pit) was dug up, all soil horizons were defined and marked with small plastic labels. Then, roots were collected arbitrarily from each horizon, revealing the layers of individual soil horizons one by one. Each soil horizon from each pit was represented by a fine root sample of approximately 100 g fresh weight. The root samples were transported to the laboratory in a cooling box and stored in labeled plastic bags in a refrigerator (4 °C) until analysis. Samples were washed under tap water and evaluated separately for each soil horizon under the microscope. Root tips from each sample were categorized into morphological groups (morphotypes) according to macroscopic criteria (Agerer 2001). Based on the morphological evaluation, morphotypes representing two contrasting types (contact and long-distance) were selected for further anatomical and molecular analysis.

Soil characteristics

We described the soil characteristics previously (Trocha et al. 2017, 2021). Briefly, the forest types of the two stands had similar characteristics and were classified as mixed broad-leaved forests. Soil types in both stands were classified as rusty and belonging to arenosols (Kabała et al. 2016).

We examined soil horizons based on soil structure in each soil pit; analysis was conducted separately for each soil horizon (soil texture and chemistry). All analyses were conducted by the Forest Management and Geodesy Agency, Poznań, Poland. The grain size composition was determined using the Casagrande

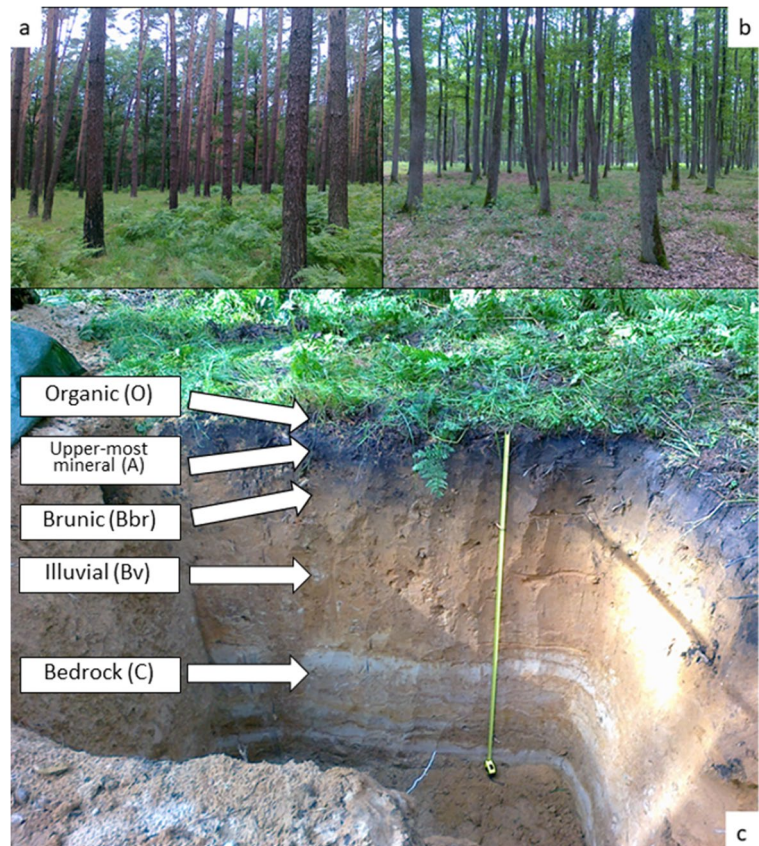
method with Proszynski's modification (according to PN- ISO 11277, 2005). The pH was measured using the potentiometric method in H_2O and KCl solution at a concentration of 1 mol dm^{-3} . The organic carbon content was assessed employing the Tyurin method, while the total nitrogen content was determined using the Kjeldahl method. The concentration of available forms of phosphorus and potassium was expressed in $\text{mg P}_2\text{O}_5$ or K_2O per 100 g^{-1} soil and was conducted using the Egner-Riehm method. The magnesium content (MgO) was determined using the Schachtschabel method. Cation exchange was assessed through flame atomic absorption spectroscopy (FAAS). *P. sylvestris* and *Q. petraea* sites were on the same sandy acid soil type. The distinguished horizons were (starting from the top horizon): organic (O, partially decomposed organic matter), uppermost mineral (A, with a greater degree of decomposition of dead organic matter), brunic (Bbr, the ongoing browning process), illuvial (Bv, the rusting process), and mother rock (C). For further analysis, we focused on organic (O), uppermost

mineral (A), brunic (Bbr), and illuvial (Bv), which are shown in Fig. 1.

Ectomycorrhizal assessment and ecm species identification

Morphotype identities were designated by molecular identification based on barcoding using ITS-rDNA sequencing. In brief, total genomic DNA was extracted from individual root tips of described morphotypes using the NucleoSpin® Plant II kit (Macherey-Nagel, Dueren Germany). The ITS-rDNA region was amplified with primers ITS1F and ITS4 (Gardes and Bruns 1993; White et al. 1990). PCRs were carried out in $15 \mu\text{L}$ reaction volumes containing $7 \mu\text{L}$ PCR AmpliMIX Taq 2x (Novazym, Poland), 0.5 mM of each primer, and $5 \mu\text{L}$ of DNA template (diluted two to 10 times) using a thermocycling profile of one cycle of 3 min at 95°C followed by 35 steps of 40 s at 95°C , 40s at 60°C , 1.5 min at 72°C , with a final step of 10 min at 72°C . PCR products were purified

Fig. 1 90-year-old, monoculture adjacent stands of Scots pine (a) and sessile oak (b) in the Zielonka Forest, which grow on rusty soils belonging to Arenosols with the same layout of horizons in the soil profile (c). The soil horizon depth for Scots pine sites was: O – 1 cm, A – 15 cm, Bbr – 24 cm, Bv – 54 cm; and for sessile oak was: O – 0.5 cm, A – 13 cm, Bbr – 36 cm, Bv – 41 cm



using exonuclease I and Fast alkaline phosphatase (Fermentas) and sequenced using a BigDye Terminator v3.1 kit and ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions. The obtained chromatographs of fungal ITS sequences were screened and edited in Chromas LITE v.2.01 (<http://www.techenysium.com.au>), after which the sequences (together with their GenBank or UNITE best matches) were aligned using a Codon-Code Aligner (<http://www.codoncode.com/aligner/>) at the $\geq 97\%$ identity threshold. The selected sequences of fungal species were deposited in GenBank (accession numbers KP731808–KP731864).

Anatomy of morphotypes

Morphotypes were fixed in a mixture of 4% paraformaldehyde (Polysciences, Warrington, PA, USA) and 4% glutaraldehyde (Polysciences, Warrington, PA, USA) in 0.1 M cacodylate buffer (Polysciences, Warrington, PA, USA) for 24 h at 4°C. Afterward, roots were washed twice in 0.05 M cacodylate buffer, dehydrated in an ascending ethanol series, and embedded in Technovit 7100 (Heraeus Kultzer, Wehrheim, Germany). Specimens were cut into 5 μm sections with a rotary microtome (Leica RM 2265, Wetzlar, Germany) and placed on microscopic slides. The cross-sections were stained in toluidine blue O in 1% tetraborate buffer to visualize the root structures and then analyzed with an

Axioskop 20 optical microscope (Carl Zeiss, Jena, Germany). Images were documented with AxioVision Rel. 4.8 software (Carl Zeiss, Jena, Germany). Anatomical traits considered in the studies were root diameter, absorptive traits (the thickness of the cortex, and mantle thickness) and stele diameter (Appendix S1). Each diameter was measured twice to more accurately determine the actual dimensions of a given root. Measured anatomical traits with their definitions were listed in Table 1.

The diameters of the xylem vessels and their cell wall thickness were also measured, and their mean radius (r) was calculated, which was used to determine the weighted average diameter of vessels in the roots (R_a) (see Formula 1) (Sperry et al. 1994):

$$R_a = 2 * \left(\frac{\sum r^5}{\sum r^4} \right)$$

Statistics

Before the analyses, the data distribution was checked using the Shapiro-Wilk test and Levene's test ($P < 0.05$). If necessary, normality of the data matrix was obtained using a logarithmic transformation. Statistically significant differences between the variants studied were determined using analysis of variance (three-factor, to evaluate the impact of fungal distance exploration type, tree species, and soil horizon; one-factor, to determine

Table 1 List of anatomical traits measured

Root trait	Definition	Rationale
Root diameter (μm)	The root diameter measured on the cross-section of the root	Responsible for nutrient absorption and resistance of water and nutrient flow
Mantle (μm)	The thickness of the mantle on the cross-section of the root	Responsible for nutrient absorption
% of mantle in diameter	The percentage of the mantle in the cross section of the root diameter	Responsible for nutrient absorption
% of absorptive area in diameter	The percentage of the mantle and cortex in the cross section of the root diameter	Responsible for nutrient absorption and resistance of water and nutrient flow
% of cortex	The percentage of the cortex in the cross section of the root diameter	Mediates the exchanged on nutrient between fungi and plant
% of stele in diameter	The percentage of the stele in the cross section of the root diameter	Mediates the conduction of water and nutrients
R_a	The average diameter of xylem vessels in the roots	The lumen to conduct water and nutrient
Cell wall thickness	The cell wall thickness of xylem vessels/trachea	Resistance to collapse of light conducting elements

the variation of root traits between soil horizons). Values were considered as statistically significant at $P < 0.05$. The means were then compared by Tukey's test at a significance level of $P < 0.05$. The plasticity index (PI) of the measured anatomical features was also counted. According to Ostonen et al. (2013), $PI = (X_{max} - X_{min}) / X_{max}$, where X_{min} is the smallest arithmetic mean and X_{max} is the largest arithmetic mean in a soil horizon. The sampling included 2 tree species $\times$ 2 exploration types $\times$ 4 soil horizons. Seven hundred and fifty-eight root cross-sections were analyzed from three soil pits, since in each sampling ca. 15 roots were evaluated. Statistical calculations were performed in JMP PRO 16 (SAS Institute Inc., Cary, NC) software.

Results

Soil structure and chemistry

Across most characteristics, the O horizon exhibited the highest values in soil samples from both

examined tree species. This trend was observed for SOM, exchange cations, and element content (C, N, P, K, Mg), O horizon had the smallest thickness (Fig. 2). The pH level increased with depth in soil horizons under *P. sylvestris*. Conversely, for *Q. petraea*, the lowest pH was observed in the mineral horizon.

Variation of absorptive root traits through soil horizons

Quercus petraea and *P. sylvestris* exhibited differences in the dominant fungal genera associated with contact exploration type ectomycorrhizas (Appendix S2). Contact exploration type was identified as being formed by *Wilcoxina* in *P. sylvestris*, whereas in *Q. petraea* was associated with *Lactarius* and *Russula* (Appendix S2). The long-distance exploration type of *P. sylvestris* was identified as *Imleria*, *Xerocomus*, *Xerocomellus*. The long-exploration type on *Q. petraea* roots was formed by symbiosis with *Scleroderma*.

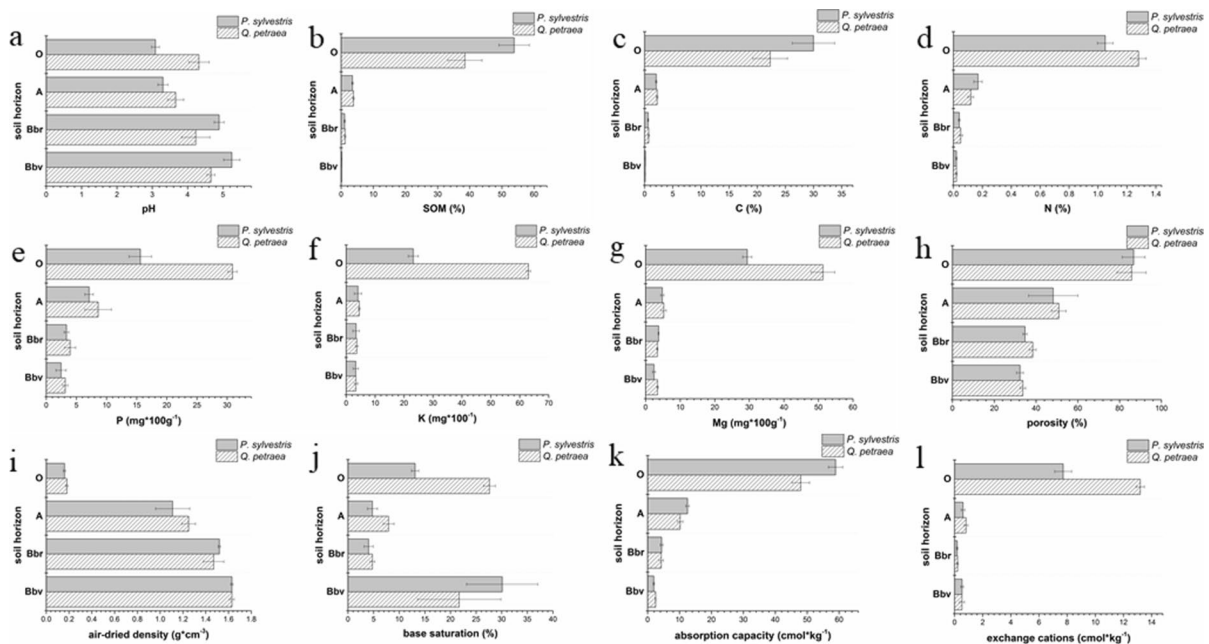


Fig. 2 Mean soil characteristics across soil horizons: organic (O), uppermost mineral (A), brunic (Bbr), illuvial (Bv) in *Pinus sylvestris* and *Quercus petraea* monoculture stands in the Zielonka Forest, Poland. All attributes were significantly different among soil horizons (ANOVA $P < 0.05$) (analysis run

for *P. sylvestris* and *Q. petraea* separately), except for pH for *Q. petraea*, which was not significantly different among soil horizons. The bar indicates the mean and the whiskers indicate the SE ($n = 3$)

Variation of absorptive root traits along soil horizons

P. sylvestris formed thicker roots compared to those of *Q. petraea* (Tables 2 and 3). On average, the roots of *Q. petraea* had a greater percentage of the absorptive area (including mantle and root cortex) within the root diameter than the roots of *P. sylvestris* (Tables 2 and 3).

The percentage of both mantle and cortex area varied among species EcM exploration type depending on the soil depth (Table 2). Mantle width tended to increase with soil depth despite overall root absorptive area (mantle + cortex) decreased in the downmost horizon within the contact exploration type of *P. sylvestris* (Table 3). The higher importance of mantle in root *P. sylvestris* root adjustment of contact type to soil depth was confirmed by PI (Table 4). The reverse trend was observed for the long-distance exploration type of *P. sylvestris* with thicker cortex formed deeper in the soil. In *Q. petraea* the proportion of absorptive area in diameter was the highest in the lower, nutrient-poor soil horizons, regardless of exploration types (Table 3).

Indeed the percentage of the cortex in root diameter on average, however, did not vary among tree species; however, roots forming the contact exploration type had a larger proportion of cortex than the long-distance exploration type (Table 2). In shallower soil horizons, both exploration types in *Q. petraea* showed a lower proportion of the cortex in root diameter. Similarly, the long-distance exploration type in *P. sylvestris* also exhibited this trend.

Changes in traits connected with the nutrients and water flow along a vertical soil profile

The PI between soil horizons indicated a higher variation for the “long-distance exploration type” than for the contact exploration type, especially for the proportion of the stele in root diameter and cell wall thickness (Table 4). This pattern was more visible for *P. sylvestris* than for *Q. petraea* (Table 2). In *Q. petraea*, the reverse trend was found for the average vessel diameter.

All three main factors (i.e., tree species, soil horizon, and exploration type) and their interactions affected the stele proportion in the root diameter (Table 2). On average, *P. sylvestris* (mean = 29.6 ± 0.68) had a higher proportion of stele

Table 2 Anova results of variation in root considering two contrasting fungal distance exploration types through soil horizon

factor	d.f.	Root diameter		% of mantle in diameter		% of cortex in diameter		% of stele in diameter		Ra		Cell wall thickness	
		F	P	F	P	F	P	F	P	F	P	F	P
Tree species (TS)	1	132.74	<0.0001	39.20	<0.0001	0.07	0.7972	44.85	<0.0001	187.63	<0.0001	188.81	<0.0001
Soil horizon (SH)	3	6.82	0.0003	1.15	0.3305	4.87	0.0030	17.04	<0.0001	2.96	0.0343	7.86	<0.0001
Exploration type (ET)	1	0.52	0.4726	52.09	<0.0001	14.65	0.0002	4.50	0.0354	0.14	0.7048	3.32	0.0706
TS × SH	1	0.38	0.7682	2.16	0.0962	7.62	<0.0001	6.51	0.0004	2.06	0.1083	4.36	0.0056
TS × ET	1	10.56	0.0014	19.64	<0.0001	3.52	0.0626	61.43	<0.0001	1.62	0.2046	10.72	0.0013
SH × ET	3	5.45	0.0014	6.90	0.0002	2.63	0.0522	11.16	<0.0001	2.68	0.0489	1.01	0.3885
TS × SH × ET	3	5.30	0.0017	6.24	0.0005	8.52	<0.0001	12.32	<0.0001	0.93	0.4280	2.09	0.1042

Statistically significant values were matched in bold and italics ($n = 3$)

Table 3 Comparison of root anatomical traits associated with nutrient absorption among soil horizons in contrasting exploration types of ectomycorrhizas of two tree species – *Pinus sylvestris* and *Quercus petraea*

Tree	EcM exploration type	Soil horizon	Diameter (μm)	Mantle width	% of mantle	% of absorptive area	% of cortex
<i>Pinus sylvestris</i>	Contact	O	374.30 (30.00)	22.49 (1.87) ab	12.71 (1.41)	71.14 (1.16) a	58.43 (2.13)
		A	414.10 (13.87)	19.97 (3.86) ab	12.88 (1.11)	66.68 (1.19) ab	61.72 (2.84)
		Bbr	386.46 (13.92)	19.73 (1.92) b	10.20 (0.89)	64.59 (1.50) b	54.38 (1.83)
		Bv	418.02 (12.05)	30.26 (3.72) a	14.47 (1.77)	67.60 (2.77) ab	53.13 (1.78)
		P	0.2841	0.0367	0.1630	0.0291	0.0720
	Long-distance	O	415.48 (30.03)	35.66 (3.21)	17.39 (1.10)	67.53 (1.95) b	50.13 (1.53) b
		A	479.55 (17.89)	41.09 (1.90)	17.29 (0.92)	73.45 (1.28) a	56.16 (1.37) a
		Bbr	428.61 (23.62)	39.93 (2.64)	18.73 (0.88)	77.05 (0.81) a	58.32 (0.79) a
		Bv	467.62 (15.73)	43.08 (2.16)	18.49 (0.86)	75.22 (1.07) a	56.72 (1.01) a
		P	0.1813	0.1257	0.6080	0.0001	0.0002
<i>Quercus petraea</i>	Contact	O	249.33 (8.35) c	27.63 (1.42)	22.23 (1.11) a	74.21 (1.00) b	51.98 (1.86) b
		A	329.15 (14.44) ab	27.56 (1.30)	16.91 (0.82) b	73.90 (0.90) b	57.00 (1.30) ab
		Bbr	298.89 (7.11) bc	25.00 (1.31)	16.88 (1.11) b	79.06 (0.80) a	62.18 (0.88) a
		Bv	396.31 (35.28) a	29.40 (3.92)	14.79 (1.19) b	76.56 (0.54) ab	61.77 (1.33) a
		P	<0.0001	0.7264	0.0002	0.0003	<0.0001
	Long-distance	O	298.97 (13.32) a	25.27 (1.72)	16.82 (0.71) b	70.20 (1.00) c	53.37 (1.09) b
		A	332.35 (12.36) a	29.86 (2.14)	17.85 (0.88) b	67.27 (1.07) c	49.42 (0.86) c
		Bbr	299.11 (16.23) a	29.79 (1.99)	19.88 (0.77) ab	74.13 (0.73) b	54.25 (0.86) ab
		Bv	243.33 (16.58) b	27.23 (3.20)	21.93 (1.01) a	79.30 (0.46) a	57.37 (1.23) a
		P	0.0012	0.3825	0.0007	<0.0001	<0.0001

Values matched with the same letter do not differ significantly (Tukey test, $P < 0.05$). Mean values were presented and SE were put into the brackets ($n = 3$). Statistically significant values were matched in bold and italics

in root diameter than *Q. petraea* (25.7 ± 0.52) roots ($P < 0.001$) (Table 2). Considering the effect of soil horizon on morphotypes, we observed a decrease in the percentage of stele in root diameter with soil depth in the long-distance exploration type within both tree species than in the contact exploration type of *Q. petraea* (Table 5). As Ra in different morphotypes varied across the soil horizons, the interaction of the exploration types with the soil horizon turned out to be significant (Tables 2 and 5). Moreover, in *Q. petraea*, the width of the cell wall tend to increase between O and Bv horizon for both morphotypes, whereas in case of *P. sylvestris* the decrease was only observed in the long-distance exploration type.

Discussion

Anatomical traits associated with absorption are driven by exploration type, modified by tree species

Exploration strategy of the ectomycorrhizas along horizons results from capabilities of specific fungal genera to utilize nitrogen at rich or nutrient-poor soil (Hobbie and Agerer 2010; Taylor et al. 2003), as well as traits variability of the most distal roots (i.e., first or second orders) that may affect their ability to forage for and take up resources from varying sources and locations (Curt and Prevosto 2003; Comas and Eissenstat 2004; Ostonen et al. 2013). Accordingly,

Table 4 Variability of anatomical parameters of roots of first root order of *Pinus sylvestris* and *Quercus petraea* and in contact and long-distance exploration type by all horizons of soil

Root trait	Plant species	Distance exploration type	CV (%)	PI
Root diameter (μm)	<i>P. sylvestris</i>	Contact	16	0.10
		Long	16	0.13
	<i>Q. petraea</i>	Contact	25	0.37
		Long	19	0.27
Mantle (μm)	<i>P. sylvestris</i>	Contact	63	0.67
		Long	20	0.17
	<i>Q. petraea</i>	Contact	26	0.21
		Long	26	0.15
% of mantle in diameter	<i>P. sylvestris</i>	Contact	31	0.29
		Long	16	0.08
	<i>Q. petraea</i>	Contact	24	0.33
		Long	17	0.23
% of absorptive area in diameter	<i>P. sylvestris</i>	Contact	8	0.09
		Long	7	0.12
	<i>Q. petraea</i>	Contact	4	0.06
		Long	7	0.15
% of stele in diameter	<i>P. sylvestris</i>	Contact	16	0.19
		Long	20	0.29
	<i>Q. petraea</i>	Contact	14	0.20
		Long	19	0.37
RA	<i>P. sylvestris</i>	Contact	15	0.21
		Long	19	0.21
	<i>Q. petraea</i>	Contact	29	0.31
		Long	27	0.22
Cell wall thickness	<i>P. sylvestris</i>	Contact	10	0.12
		Long	13	0.20
	<i>Q. petraea</i>	Contact	16	0.18
		Long	17	0.22

Coefficient variation (CV) was calculated considering all horizons, while plasticity index (PI) was determined considering mean trait values for soil horizons

the exploration and nutrient uptake in the upper and down soil horizons may be related to different strategies resulted in either morphological and biomass adjustment of absorptive roots or changes in anatomy of different morphotypes to varied resources availability (Francini and Sebastini 2010; Ostonen et al. 2013). We observed that increase of absorptive area in long-distance exploration type resulted from wider cortex in deepest soil profile, which supports our hypothesis. As at greater depth is harder to produce more roots but edaphic conditions make that these roots are thicker (Prieto et al. 2015). Under such conditions capture of soil resources may be a result of positive correlation between the abundance of thick absorptive fine roots and the proportion of long-distance exploration types (Chen et al. 2018a, b).

Consequently, *P. sylvestris*, characterized by higher diameter absorptive roots should benefit from the long-distance exploration by acquiring resources well beyond the deepest depletion zones at greatest depth. Long-distance exploration type of *P. sylvestris* benefits from the wider cortex, as a such pattern increases the exchange area between their hydrophobic fungal hyphae and the tree at greatest depths. Conversely, to acquire sufficient amount of resources *P. sylvestris* contact exploration types are characterized by a wider mantle and not thicker cortex. It remains possible that increased mantle size could result from higher demand for N among the ectomycorrhizal fungi forming the contact exploration type in *P. sylvestris* to maintain fungal metabolic requirements. What is more, proportion of cortex within contact

Table 5 Comparison of root anatomical traits associated with water absorption among soil horizons in contrasting exploration types of ectomycorrhizas of two tree species – *Pinus sylvestris* and *Quercus petraea*

Tree	EcM exploration type	Soil horizon	% stele	Ra	Cell wall thickness
<i>Pinus sylvestris</i>	Contact	O	28.86 (1.16) b	7.83 (0.35) ab	1.36 (0.03)
		A	33.32 (1.19) ab	9.51 (0.50) a	1.55 (0.07)
		Bbr	35.41 (1.50) a	8.25 (0.30) ab	1.44 (0.05)
		Bv	32.40 (2.77) ab	7.48 (0.49) b	1.45 (0.07)
		P	0.0276	0.0406	0.1521
	Long-distance	O	32.47 (1.95) a	8.97 (0.31) a	1.55 (0.06) a
		A	26.55 (1.28) b	7.45 (1.46) b	1.43 (0.05) ab
		Bbr	22.95 (0.81) b	7.10 (1.20) ab	1.24 (0.02) b
		Bv	24.78 (1.07) b	7.56 (0.61) ab	1.37 (0.06) b
		P	<0.0001	0.0396	0.0240
	Contact	O	25.79 (1.00) a	3.61 (0.28) b	0.89 (0.05) b
		A	26.10 (0.90) a	5.19 (0.32) a	1.09 (0.04) a
		Bbr	20.94 (0.80) b	4.78 (0.21) ab	0.92 (0.02) b
		Bv	23.44 (0.54) ab	4.52 (0.35) ab	1.03 (0.03) ab
		P	<0.0001	0.0214	0.0006
<i>Quercus petraea</i>	Long-distance	O	29.80 (1.00) a	4.58 (0.58)	0.98 (0.09) b
		A	32.73 (1.07) a	5.31 (0.28)	1.25 (0.04) a
		Bbr	25.87 (0.73) b	4.72 (0.61)	1.05 (0.04) ab
		Bv	20.70 (0.46) c	4.12 (0.32)	1.16 (0.07) ab
		P	<0.0001	0.2186	0.0139

Values matched with the same letter did not differ significantly (Tukey test, $P < 0.05$). Mean values were presented and SE were put in the brackets ($n = 3$). Statistically significant values were matched in bold and italics

exploration type varied between *P. sylvestris* and *Q. petraea* stressing that under the same environmental conditions anatomy is more species-dependent. In the contact morphotype of *P. sylvestris*, which is formed by hydrophilic hyphae that facilitate water movement, an increase in cortex width may not necessarily results in a corresponding enhancement of the uptake capacity. On the other hand, contact type roots of *Q. petraea* were characterized by wider cortex to enhance surface of nutrient exchange between plant and fungus - angiosperm form Hartig net only within one layer of cortex. Indeed, Liu et al. (2023) indicated that angiosperms may adjust their cortex area to flexibly increase absorption or facilitate more efficient transport of resources even when not associated with fungal species forming mycorrhizas, in contrast to gymnosperms, which tend to be more uniformly reliant on mycorrhizas. Accordingly, the functional changes in anatomy of *Q. petraea* absorptive roots suggest better/more consistent adjustment to balance absorption across soil horizons. Therefore, as cortex thicknesses was found to be less variable in oak

roots, even in different morphotypes, oak roots adapt similar strategies to absorb nutrients in nutrient-poor soil which can include fungal uptake of nutrients from various sources, completely exhausting niches in the soil. Support for our assumption comes from previously indicated similarity of N acquisition by oaks between contact and long-distance exploration types in nutrient-poor horizons (Fig. 2, Appendix S3). However, we cannot exclude the possibility that the observed physiological effect results from an interaction between anatomical adjustment and specific fungal genera in *Q. petraea*. Irrespectively that higher-biomass exploration types dominate in soil of lower nitrogen availability (Hobbie and Agerer 2010), while contact types dominate in soils with nitrogen amendment nitrophilic ectomycorrhizal fungi (Morison et al. 2016), indicated that similar N level in oaks roots along soil profile may be due to the development of different foraging strategies (Chen et al. 2013, 2016) within specific mycorrhizal fungi based on specific functional characteristics (Chen et al. 2016). This is confirmed by fungal species forming contact

exploration type *Lactarius* and *Rusulla* Nygren et al. 2008) and *Scleroderma* (Trocha et al. 2021) efficiently utilize different nitrogen sources, both organic and inorganic. Thus, deeper rooting may have profound effects on the ecological processes in the soil horizons due to the close links between roots and soil organic matter storage, nutrient availability, and microbial activity (Jobbágy and Jackson 2000; Joslin et al. 2006; Iversen et al. 2008).

Tree species drive anatomical traits associated with water and nutrient resource flow

We found that the anatomical traits associated with water and nutrient uptake are under robust species control since there were no within-species differences in root diameter, Ra, and cell wall thickness in relation to different exploration types. Between species, *P. sylvestris* was characterized by a larger diameter of absorptive roots compared to the roots of *Q. petraea*. Although we expected a decrease in first-order root diameter as a function of adjustment to more efficient water uptake from deeper soil horizons, we only found such a trend for *Q. petraea* roots with long-distance exploration type.

Generally, the vessels evolved in woody angiosperms usually have a higher hydraulic conductance than gymnosperms for transporting water through the tracheids (Esau 1977). Thus, the larger vessels observed in *Q. petraea* at greater soil depths are likely adapted for efficient water transport. Our results are in line with previous studies, showing that deep roots had vessels with larger diameters than shallow roots, up to a depth of 18–20 m (McElrone et al. 2004), with higher hydraulic conductivity in subsurface soil layer (Wang et al. 2019). While wider vessels in deep roots may be associated with greater vulnerability to xylem embolism (e.g., McElrone et al. 2004); the risk is mitigated in deeper soils, where extreme temperatures and freeze-thaw cycles are less frequent (Coleman et al. 2004). Mechanically, the vessel size patterns in *Q. petraea* suggest an adaptation to access deep subsoil and bedrock water during droughts, ensuring sustained transpiration (Querejeta et al. 2007; Voltas et al. 2015) due to the relative stability of water availability in bedrock (Ryel et al. 2008). Thus, *Q. petraea*, similar to *Q. robur*, may have greater reliance on deep water resources (Zadworny et al. 2021) and as has been noted in deep-rooted species (Jobbágy

and Jackson 2001; Sardans and Peñuelas 2014), the vessel diameter should be adjusted across soil horizons (Caldwell et al. 1998; Bauerle et al. 2008). Furthermore, as contract exploration type take up nutrient by surface, increased water uptake by hyphae may be less significant when soil is near saturation, as root surfaces are already in direct contact with water (Lehto and Zwiazek 2011).

Conclusions

Given that absorptive roots play a crucial role in tree growth, function, and nutrient cycling (Gower et al. 1996; Gill and Jackson 2000), our findings provide insights into resource acquisition strategies across soil profiles. We show that root exploration types significantly influence responses to microenvironmental variations in soil horizons shaped by water and nutrient availability. Long-distance exploration types exhibited greater anatomical adaptation across soil depths, notably by increasing cortex thickness to access nutrients in nitrogen-poor horizons. Roots with long-distance exploration type consistently increased cortex thicknesses to obtain nutrients from nitrogen poor soil horizons in both tree species. In contrast to *P. sylvestris*, the absorptive area in *Q. petraea* was less dependent on ectomycorrhizal (EcM) exploration type. This suggests that long distance type engage higher carbon allocation to build a space for fungus-root establishment and functioning, and that roots of fast growing species are more plastic in absorptive traits adjustment to specific horizon whereas slow growing *Q. petraea* rely more on nutrient and water absorption irrespectively of soil horizons i.e. observed tendency to increase mantle width, percentage of absorptive and cortex area in both morphotypes. Contrary, diverse pattern of mantle width in contact exploration types is an evidence suggesting that fungal communities also facing the changing environmental habitats more depending on their physiology. Thus fungal species forming contact exploration type inhabiting the roots produced in the deepest soil horizon should prioritize intensive exploration of surrounding soil by forming dense mantle, whereas long distance morphotypes of higher biomass investment (thicker cortex) may persist longer time and because of are widely dispersed. Finally, fine root anatomy in deeply rooted *Q. petraea* showed an

increase in vessel diameter and thicker vessel walls, likely to enhance water uptake and protect against mechanical pressure in deeper horizons—adaptations not observed in the pioneer species *P. sylvestris*.

Together, these findings offer new insights into the nutrient and water acquisition patterns of different root morphotypes across soil horizons. However, further research is essential to evaluate how nutrient and water uptake vary at different soil depths, which is critical for a deeper understanding of fine root function and the unique contributions of their fungal partners.

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Data availability Data is available upon request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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