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Does a fungal species drive ectomycorrhizal root traits in *Alnus* spp.?

Ivika Ostonen, Leho Tedersoo, Triin Suvi, and Krista Lõhmus

Abstract: Ectomycorrhizal (EcM) fungi contribute significantly to the shaping of short-root morphology, playing an important role in balancing the costs and benefits of root growth and nutrient uptake and exchange in boreal forests. We aimed to assess the effect of various EcM fungal taxa on root traits at seven sites dominated by grey alder, *Alnus incana* (L.) Moench, and black alder, *Alnus glutinosa* (L.) Gaertn. Mean root size, specific root length, specific root area, root tissue density, and root-tip frequency of EcM short roots were measured in EcM anatomotypes in relation to the effects of host species, soil moisture level, and nutrient status. Redundancy analysis revealed that anatomotype, alder species, site, and soil parameters (N, P, K, Ca, and Mg concentrations, pH, organic-matter content) accounted for 42.3% ($p < 0.001$) of the total variation in EcM root morphology. Variation decreased in the following order: anatomotypes (27.9%) > soil parameters and sites (19.9%) > alder species (5.1%). EcM fungus species had the primary influence on EcM short-root size. EcM roots of the dominant anatomotype, *Alnicola* spp., had the highest specific root length and specific root area in both alder species. Short-root morphology depends most strongly on the fungal taxa involved, which indicates that the type of mycobiont has an important influence on the functional properties of fine roots.

Résumé : Les champignons ectomycorhiziens (EcM) contribuent de manière significative à déterminer la morphologie des racines courtes, jouant ainsi un rôle important dans le maintien de l'équilibre entre les coûts et les bénéfices associés à la croissance racinaire et à l'absorption ainsi qu'aux échanges dans les forêts boréales. Nous visions à évaluer l'effet des taxons des champignons EcM sur les caractéristiques des racines dans sept stations dominées par *Alnus incana* (L.) Moench et *Alnus glutinosa* (L.) Gaertn. La dimension moyenne des racines courtes mycorhizées, la longueur spécifique des racines (LSR) et la surface spécifique des racines (SSR) ont été mesurées chez les types anatomiques d'EcM en relation avec les effets de l'espèce hôte, de l'humidité du sol et du statut des nutriments. L'analyse de redondance a révélé que le type anatomique, l'espèce d'aulne, la station et les paramètres du sol (N, P, K, Ca, Mg, pH et matière organique) expliquent 42,3 % ($p < 0{,}001$) de la variation totale de la morphologie des racines mycorhizées. La variation décrite diminuait dans l'ordre suivant : types anatomiques (27,9 %) > paramètres du sol et stations (19,9 %) > espèces d'aulne (5,1 %). Le champignon EcM influençait surtout la dimension des racines courtes mycorhizées. Les racines mycorhizées par le type anatomique dominant, *Alnicola* spp., avaient les valeurs de LSR et de SSR les plus élevées chez les deux espèces d'aulne. La morphologie des racines courtes dépend avant tout des taxons fongiques impliqués, ce qui indique que le type de mycobiontes a une grande influence sur les propriétés fonctionnelles des racines fines.

[Traduit par la Rédaction]

Introduction

Ectomycorrhizal (EcM) associations play a key role in mineral nutrition of Pinaceae, Fagaceae, Betulaceae, and Salicaceae in boreal and temperate forest ecosystems (Brundrett 2002). EcM short roots with primary structure are responsible for water and nutrient uptake and it is well known that the formation of EcM roots involves alterations in the morphology of the plant root and fungal mycelium. Morphological and anatomical features of ectomycorrhizas are more or less characteristic of the fungal species forming the anatomotype (Agerer 1987–1998). Considerable variation in a range of physiological functions, such as enzyme activities (Courty et al. 2005), nutrient uptake (Abuzinadah and Read 1989; Perez-Moreno and Read 2000; Landeweert et al. 2001; Chalot et al. 2002), and heavy-metal tolerance (Jentschke and Godbold 2000), has been shown among EcM species. There could be great differences in EcM effectiveness in facilitating tree growth (Burgess et al. 1993). EcM diversity and community structure are related to the growth rate of the host (Korkama et al. 2006). EcM fungi can explore the surrounding soil by means of extramatrical mycelia, which are either concentrated close to the mycorrhizal mantle or form far-reaching rhizomorphs of different exploration types, and each EcM fungus (or exploration type) may utilize a distinct foraging strategy (Agerer 2001).

Owing to improvements in species-identification methodology (Horton and Bruns 2001), the number of studies focusing on EcM fungi and EcM community structure in different sites and hosts has increased. Much less is known about the effects of different anatomotypes (and (or) EcM fungus species) on EcM root morphology and about EcM

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community structure in relation to ecosystem function, particularly mineral nutrition (Godbold 2005).

However, community structure of EcM fungi is an important biotic factor, with a significant effect on EcM root morphology, together with abiotic factors (e.g., physical and chemical characteristics of soil) at the site, and genetic variability within host trees. Different functional parameters of short EcM roots, e.g., specific root area (SRA), specific root length (SRL), and root tissue density (RTD), have been used as cost–benefit indices at the level of both the individual root and the entire root system, assuming that resource acquisition is proportional to root length or surface area, and that root cost (construction and maintenance) is proportional to mass (Fitter 1991; Pregitzer et al. 2002; Withington et al. 2006; Ostonen et al. 2007*a*, 2007*b*). However, EcM short-root parameters can be separated into independent variables — mean length (*L*), diameter (*D*), and RTD — and dependent or derived variables calculated from the former variables SRA and SRL, and root-tip frequency per unit dry mass (RTFW) and root-tip frequency per unit length (RTFL) (Ostonen et al. 2006). The larger the SRA and the smaller the RTD, the more effective is the strategy of allocating assimilates to the build-up of EcM roots (Ostonen et al. 1999; Wahl and Ryser 2000). At the level of the entire root system, optimality theory predicts that a tree optimizes root function by maximizing its nutrient and water uptake with minimum resource investment in the root system (Leuschner et al. 2004).

The impact of fungal symbionts on EcM root characteristics is reported to be significant in Norway spruce; differences in the volume proportions of mantle and cortex in the root, as well as in SRA and RTD, have been revealed (Ostonen et al. 1999; Ostonen and Lõhmus 2003). In the rhizosphere, Lõhmus et al. (2006) showed a positive correlation between SRA of EcM roots and the functional diversity and activity of microbial communities at the soil–root interface and in the bulk soil. At the level of the forest ecosystem, a positive correlation between SRA of EcM roots and the assimilation efficiency of leaves ($kg \cdot kg^{-1} \cdot year^{-1}$) in alders has been reported (Lõhmus et al. 2006).

Alder species are indigenous to a large part of Europe. They occur in a wide variety of habitats ranging from dry to wet, and in soils ranging from from calcareous to acidic. However, while black alder (*Alnus glutinosa* (L.) Gaertn.) is more commonly the dominant tree species in forests in wetlands, both black alder and grey alder (*Alnus incana* (L.) Moench.) forests are typical of the riparian zone, and grey alder also tolerates relatively dry soils.

Grey alder and black alder were selected as model taxa in our study because EcM colonization of alders responds strongly to changes in soil conditions (Baar et al. 2000; Dilly et al. 2000), and alders host a limited set of EcM fungi, which allows comparisons to be made among and between taxa at several geographical scales (Pritsch et al. 1997*a*, 1997*b*; Becerra et al. 2005).

Our background hypothesis was that the different anatomotypes of EcM roots resulting from colonization of the same host by different fungi have significant differences in their morphological parameters. However, the pattern of the EcM community depends on both forest site type and soil nutrient availability (Toljander et al. 2006; Lilleskov et al. 2008), and differences in the proportions of the dominant anatomotypes may eventually be exhibited in the mineral nutrition of trees at different sites. If this is the case, then relating EcM community structure and colonization to the morphological parameters of EcM roots in boreal forests, to account for mineral-nutrition efficiency and (or) strategy, would increase our understanding of the function of EcM community structure in the mineral nutrition of boreal-forest ecosystems.

We studied the impact of EcM community structure on EcM root morphology in grey and black alder stands growing in Estonian forest sites where soil conditions and previous land use varied. Our main aim was to elucidate how the morphological parameters of EcM roots depend on EcM fungus species, site and soil conditions, and host tree species.

Material and methods

The study was performed in three grey alder and four black alder stands (Table 1) situated throughout Estonia. The sites were selected to cover different soil conditions, to ensure a wide spectrum of EcM fungus species colonizing alder roots. Previous land use was taken into consideration. Two 29-year-old black alder plantations (Songa and Sirgala) originated from the same set of mother trees (Vares et al. 2004). The Järvselja site is situated in a primeval mixed forest in southeast Estonia that has remained unmanaged for >200 years. Wet plots were established in a forest area in a minerotrophic black alder swamp and dry plots in an adjoining *Vaccinium myrtillus* – spruce forest where black alder forms a subdominant tree layer (Tedersoo et al. 2008). The Inka black alder and Porijõe grey alder study areas (Mander et al. 2008) are in the flood plain of small rivers that host a natural, unmanaged alder stand. The undisturbed grey alder forest at Saka lies on a talus slope of the North Estonian (Ordovician) Klint, where the soils on Cambrian clays are influenced by carbonate-rich ground water from limestone terraces (Rooma and Paal 2001). The Leidisoo grey alder forest is situated on the bank of a young peat bog in western Estonia, where the soils are influenced by contrasting hydrological conditions and calcareous bedrock; pH_{KCl} variation within the site is almost 3 units (Table 1).

At all sites, three plots were established in a relatively dry area and three in a wet area with substantial seasonal waterlogging. Soil moisture status was estimated qualitatively.

Root sampling and processing

EcM roots were sampled in alder stands in September 2005 at Porijõgi, in September 2006 at Songa, and in September 2007 at the other five sites. In each plot, three root samples (15 cm × 15 cm to 10 cm depth) were collected using a flat spade. The root fraction was cleaned of adhering soil and clay by careful rinsing in tap water. Roots were placed in large Petri dishes filled with water and identified to tree species according to the presence and shape of EcM and nodules. The EcM roots of alders were separated into anatomotypes (Agerer 1991) using a light microscope equipped with Normarski differential interference contrast at 1000× magnification, scored for relative abundance, and subsequently identified using sequence analysis of nuclear

Table 1. Site and soil characteristics of the alder stands.

Site	Site type	Age (years)	Soil type	pH_{KCl}	N (%)	P ($mg \cdot kg^{-1}$)	K ($mg \cdot kg^{-1}$)	Ca ($mg \cdot kg^{-1}$)	Mg ($mg \cdot kg^{-1}$)	Organic-matter content (%)
Alnus incana										
Leidisoo (59° 07′N, 23°40′E)	Swamp forest on bank of young peat bog	40	Histic gleysol	4.8	1.41	41.2	152	5072	299	32
Saka (59°26′N, 27°12′E)	Coastal swamp forest on talus slope of Ordovician Klint	50	Calcaric–gleyic Leptosol	6.5	0.33	148.4	126	3063	524	7
Porijõe (58°13′N, 26°47′E)	*Aegopodium*, natural forest land	28	Mollic Gleysol	7.0	0.53	48.7	141	3113	569	11
Alnus glutinosa										
Inka (59°07′N, 23°52′E)	Swamp forest on maritime sand and calcaric bedrock	85	Histic Fluvisol	4.6	1.42	19.6	151	4662	320	33
Songa (58°19′N, 25°21′E)	*Aegopodium* sp., natural forest land	29	Mollic Gleysol	4.6	0.89	34.7	181	3003	583	21
Järvselja (58°17′N, 27°19′E)	*Calla* sp., primeval forest	140	Sapric histosol	5.5	2.55	101.3	488	6483	1841	78
Sirgala (59°17′N, 27°44′E)	Reclaimed forest on alkaline oil-shale mining area	29	Calcaric Regosol	6.8	0.35	31.1	143	4294	325	10

Note: Forest site types follow Paal (1997). Soil characteristics in the 0–10 cm layer are as follows: soil type (FAO-UNESCO 1994), pH_{KCl}, Kjeldahl nitrogen (N), available (lactate soluble) phosphorus (P), available potassium (K), exchangeable calcium (Ca) and magnesium (Mg), and loss on ignition (LOI) as organic-matter content.

rDNA internal transcribed spacer (ITS) and large subunit regions. Sequencing was performed with the primer ITS5 (5′-ggaagtaaaagtcgtaacaagg-3′). At polymorphic sites, reverse sequencing with the primer ITS4 (5′-tcctccgcttattgatatgc-3′) or LF340 (5′-tacttgtkcgctatcgg-3′) was also run to improve sequence quality. Large subunit regions were amplified in most species using the primers ctb6 (5′-gcatatcaataagcg-gagg-3′) and (or) LR5 (5′-tcctgagggaaacttcg-3′). Sequences were assembled, trimmed, checked, and edited in SEQUENCHER 4.5 software (GeneCodes Corp., Ann Arbor, Michigan) and 97% ITS region identity was used as the barcoding threshold. A more detailed description of processing molecular typing and DNA barcoding of *Alnus*-associated ectomycorrhizal fungi on root tips at the seven studied sites is presented in Tedersoo et al. (2009).

A random EcM root subsample was taken from each anatomotype sample. The sets of 5–113 subsamples per anatomotype totalled 50–1150 tips throughout all stands. We acquired more subsamples from dominant anatomotypes. The mean values of the morphological parameters in each stand also reflected the proportions of the different anatomotypes. EcM roots were mostly only first order; however, some anatomotypes included ramified EcM roots of the first and second orders. The root tips were washed with tap water to remove soil particles and then cleaned with a small soft brush to remove all remaining soil particles. Root tips were counted under a microscope. EcM root diameter, length, and projection area were measured with WinRHIZO™ Pro 2003*b* (Regent Instruments Inc., Québec, Quebec). The air-dry roots were dried at 70 °C for 2 h to constant mass and then weighed.

Morphological parameters such as mean D (mm), L (mm), and (W (mg), root-tip volume (V, mm^3), SRA ($m^2 \cdot kg^{-1}$), SRL ($g \cdot m^{-1}$), RTD ($kg \cdot m^{-3}$), RTFW (number·(mg dry mass)$^{-1}$) and RTFL (number·cm^{-1}) were measured or calculated (Ostonen et al. 2007*a*) separately for each anatomotype.

Soil sampling

Approximately 50 g of rhizosphere soil was carefully separated from roots, pooled by plot, and placed in plastic bags according to the rooting depth of each sample in each plot. Soil samples were dried at 70 °C and mineral nutrients were analyzed. Total N was determined by the Kjeldahl method with a Tecator ASN 3313. P was extracted by 15 ammonium lactate and measured by flow-injection analysis. Available K was determined from the same solution by the flame photometric method (AOAC956.01). Exchangeable Ca and Mg in the soil were measured from ammonium acetate extract (pH 7.0). Organic-matter content was determined on the basis of LOI for 2 h at 360 °C. Soil pH was measured in 1 mol/L 20 potassium chloride solution.

Statistical methods

The normality of short-root variables for different tree species in a site and for five dominant anatomotypes was checked by means of Lilliefors and Shapiro–Wilk tests. The variables were normally distributed except for RTFW and RTFL, which were log-transformed. Rare anatomotypes, representing one to four samples per stand, were used only for analyzing differences between stands and tree species and were excluded from the analysis between anatomotypes.

Multiple comparison of means was applied using Tukey's test for unequal sample sizes and 95% confidence intervals. The software STATISTICA 7.0 was used; the significance level $\alpha = 0.05$ was accepted in all cases.

The effects of site, tree species, anatomotype, and soil nutrient variables on morphological parameters of EcM roots were studied using three-way mixed ANOVA for each root parameter separately, with plot as a random factor nested within site, and site nested within host species. Soil pH and nutrient concentrations were used as covariates. The five most frequent anatomotypes — *Alnicola* spp., *Tomentella* aff. *sublilacina*, *Tomentella* aff. *ellisii*, *Lactarius cyathuliformis*, *Genea–Humaria* lineage sp. — were included in the analysis. Morphological parameters of EcM roots and soil nutrient concentrations ($mg \cdot kg^{-1}$) were log-transformed and soil nutrient concentrations (%) were arcsine-square-root-transformed prior to the analyses to meet the assumptions of parametric tests.

Principal component analysis and redundancy analysis (RDA) (CANOCO program; ter Braak and Šmilauer 2002) were used to detect and visualize relationships between root characteristics and anatomotypes, and between site and soil conditions and tree species. The significance of RDA results was tested with a permutation test ($p < 0.01$).

Results

Sources of variation in EcM root morphology

The results of principal component analysis for all measured and calculated short-root characteristics showed that two axes accounted for 81% of the total variation in short-root traits. According to the RDA, all factors — anatomotype, alder species, site, and soil parameters (N, P, K, Ca, Mg concentrations, pH, organic-matter content) — accounted for 42.3% ($p < 0.001$) of the total variation in short-root morphology (Fig. 1). The described variation in short-root morphology decreased in the following order: anatomotypes (27.9%) > soil parameters and sites (19.9%) > alder species (5.1%). Even when rare anatomotypes (<5% at any site) were removed, the dominant types still explained 26.9% of total variation in short-root morphology (Fig. 1).

Anatomotypes

The number of different anatomotypes and molecular species of EcM fungi differed between alder stands, amounting to 40 species in total. The smallest number of different EcM fungal species was found in Sirgala and the highest number in Porijõe, 11 and 18 molecular species, respectively. A detailed description of the community composition of *Alnus*-associated EcM fungi on root tips at the seven studied sites is presented in Tedersoo et al. (2009). In all studied alder forests, the anatomotypes *Alnicola* spp. and *T.* aff. *sublilacina* dominated, ranging from 65% to 85% of all detected anatomotypes (Table 2). The dominant anatomotypes possessed similar short-distance exploration types with regard to their extrametrical mycelia (Agerer 2001). *Alnicola* spp. comprised several closely related molecular species that could not be distinguished by anatomotyping. However, there was no clear trend in EcM root morphology among species within the anatomotype group *Alnicola* spp. In the RDA ordination, *Tomentella* species tended to diverge according to root morphological parameters (Fig. 1). Anatomotype was the main factor determining the diameter, length, and mass of EcM short roots in alder species, as revealed by three-way ANOVA (Table 3). The mean root diameter in *L. cyathuliformis* was significantly larger than in *Alnicola* spp. and *T.* aff. *sublilacina* (Table 4). *Genea–Humaria* lineage sp. and *Paxillus filamentosus* differed significantly from other anatomotypes in being shorter in grey and black alder stands, respectively (Fig. 1, Table 4).

According to the results of three-way mixed ANOVA, all studied main factors — anatomotype, host tree species, site, and their interactions — had a significant effect on SRA and SRL (Table 3). Of the main effects, only host tree species and site significantly influenced RTD of EcM short roots. However, host species × anatomotype and site × anatomotype interactions also had a significant impact on RTD of EcM short roots (Table 3). SRA and SRL were the highest and RTD was the lowest for EcM short roots infected by *Alnicola* spp. (Fig. 1, Table 4) for both alder species. In *T.* aff. *sublilacina*, mean RTD was up to 1.3–1.5× higher for both alder species than for other dominant anatomotypes (Table 4). Mean RTFW was significantly higher in *Alnicola* spp. than in *T.* aff. *sublilacina* for both alder species (Table 4).

Tree species

Short roots of black alder were longer and heavier than those of grey alder (*t* test, $p < 0.05$). EcM short roots colonized by *Alnicola* spp. and *T.* aff. *sublilacina* were significantly longer in black alder stands (Table 4). However, according to the results of three-way mixed ANOVA, the effect of alder species on the length of EcM short roots was insignificant (Table 3). The functional characteristics SRA and SRL were significantly higher in grey alder EcM short roots than in those of black alder (Table 5). Alder species had a significant effect on SRA, SRL, and RTD of the dominant anatomotype: SRA and SRL were higher in *Alnicola* spp. and RTD was lower in *A. incana* (Table 4).

Site and soil conditions

EcM root parameters did not differ significantly between waterlogged and dry plots, either for all anatomotypes combined or for different anatomotypes separately.

According to RDA, site and soil characteristics together accounted for 19.9% of total variability in morphological parameters of EcM roots. Soil characteristics alone accounted for 15.7%, most of which (12.7%) was due to N (%), P ($mg \cdot kg^{-1}$), K ($mg \cdot kg^{-1}$), and organic-matter content.

Site significantly affected the functional characteristics SRA, SRL, and RTD (Table 3). RTD was affected most strongly by site, and soil Mg concentration (Table 3) correlated positively with RTD ($r = 0.36$, $p < 0.0001$). However, for two dominant anatomotypes the correlation between RTD and soil Mg concentration was significantly positive only for *Alnicola* spp. ($r = 0.43$, $p < 0.0001$); no statistically significant correlation for RTD of *T. sublilacina*-colonized EcM roots was found. According to three-way mixed ANOVA, *D* and *L* of EcM short-roots were significantly affected by percent soil N; in the case of *L*, soil P concentration and LOI were even more important (Table 3).

Fig. 1. Ordination diagram of redundancy analysis indicating the relative importance of ectomycorrhizal (EcM) short-root traits (arrows) in relation to EcM anatomotypes (▲), sites (●, *Alnus glutinosa*; ○, *Alnus incana*), and tree species (■, *A. glutinosa*; □, *A. incana*). Abbreviations: *D*, EcM short-root diameter (mm); *W*, EcM short-root-tip dry mass (mg); *V*, EcM short-root-tip volume (mm^3); *L*, EcM short-root-tip length (mm); RTD, root tissue density ($kg \cdot m^{-3}$); SRA, specific root area ($m^2 \cdot kg^{-1}$); SRL, specific root length ($m \cdot g^{-1}$); RTFW, EcM short-root-tip frequency per unit dry mass ($number \cdot mg^{-1}$); RTFL, EcM short-root-tip frequency per unit length ($number \cdot cm^{-1}$).

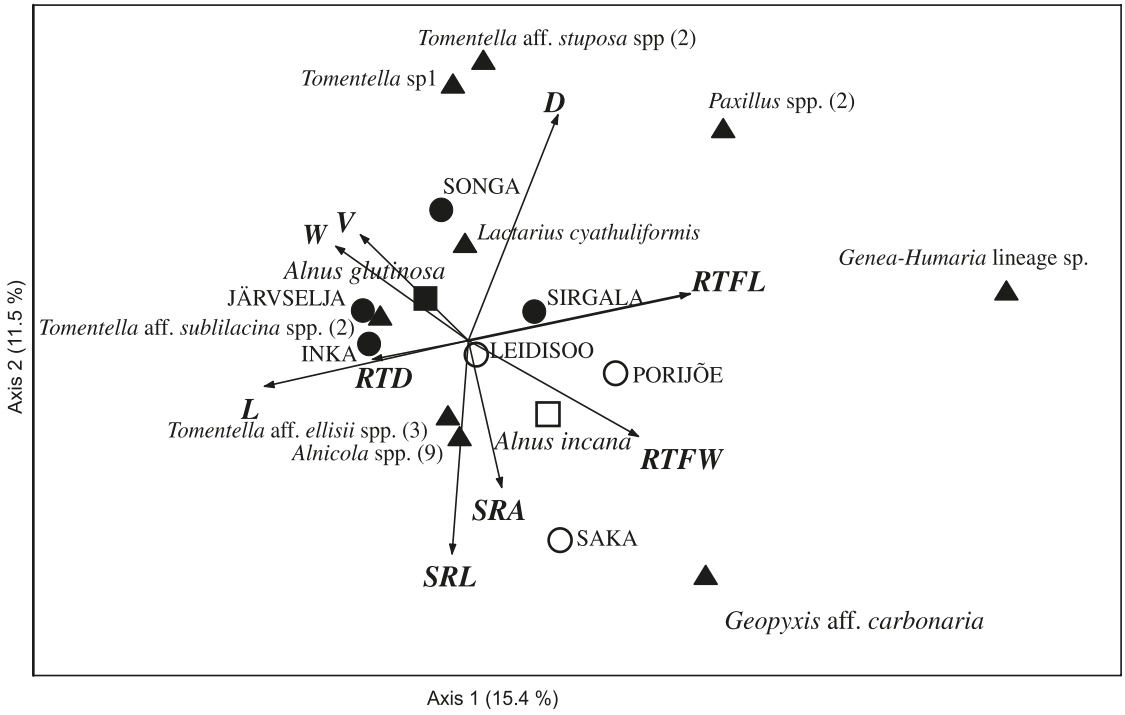

Table 2. Abundance (%) of the most common EcM anatomotypes used for determining root traits in *Alnus incana* and *A. glutinosa* stands in seven sites.

	Alnus incana			*Alnus glutinosa*			
	Leidisoo	Saka	Porijõe	Inka	Songa	Järvselja	Sirgala
Alnicola spp.	48	44	55	55	41	46	34
Tomentella aff. *sublilacina*	29	22	25	21	24	39	32
Tomentella aff. *ellisii*	5	16	<1	3	25	0	2
Lactarius cyathuliformis s.l.	5	2	4	8	6	<1	0
Genea–Humaria lineage sp.	2	5	7	<1	0	0	0
Tomentella sp. 1	7	0	0	2	<1	5	0
Tomentella aff. *stuposa*	1	<1	<1	4	0	2	11
Paxillus filamentosus	0	3	<1	0	0	0	13
Geopyxis cf. *carbonaria*	0	4	0	<1	<1	0	7

Values for the same host species also differed significantly among sites (Table 5). In grey alder stands, mean *D* was lower at Saka than at Leidisoo and Porijõe. The older natural black alder stands at Inka and Järvselja had significantly longer, thinner roots than the younger planted stands at Sirgala and Songa (Table 5). However, grey and black alder stands were grouped differently by the functional parameters SRA and RTD than by *D* and *L* (Table 5).

Discussion

The mycorrhizal structures of EcM roots of *Alnus* spp. have been extensively analyzed from the mycological perspective (Brunner and Horak 1990; Brunner et al. 1992; Pritsch et al. 1997*a*, 1997*b*; Becerra et al. 2005; Tedersoo et al. 2009). Data on the effect of EcM fungus species and community structure on short-root morphology and eventually on the mineral nutrition of trees are still scarce because identification techniques have only recently become available and because of difficulties in culturing most EcM fungi. In this study, we show that EcM anatomotype is the most important factor determining short-root traits in the two *Alnus* species, which confirms the postulated differences in the ecological functions of EcM fungal taxa below ground. In particular, the present results demonstrate that the particular EcM fungus strongly affects the length, mass, and diameter of root tips. However, *D* values for EcM short roots varied less than *L* values in all cases, because variation in *D* values on elongation of root cells; differences in *L* values involve

Table 3. *F* statistics and *p* values for the effects of site, host tree species, anatomotype, soil moisture level, their interactions, and soil variables on EcM short-root traits as revealed by three-way mixed ANOVA.

		D (mm)		*W* (mg)		*L* (mm)		RTD (kg·m^{-3})		SRA (m^2·kg^{-1})		SRL (g·m^{-1})	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Site	1	1.1	0.301	0.5	0.505	1.6	0.207	26.0	**<0.001**	16.5	**<0.001**	8.7	**0.003**
Alnus sp.	1	14.4	**0.001**	1.0	0.331	3.0	0.085	5.3	**0.023**	11.2	**0.001**	16.8	**0.003**
Anatomotype	4	14.3	**<0.001**	3.2	**0.015**	2.9	**0.024**	1.2	0.297	3.8	**0.004**	9.1	**<0.001**
Soil moisture level	1	2.4	0.127	0.1	0.811	0.6	0.426	0.1	0.928	0.8	0.370	1.1	0.304
N	1	4.2	**0.041**	—	ns	4.3	**0.039**	—	ns	—	ns	—	ns
P	1	—	ns	—	ns	14.6	**0.001**	—	ns	3.9	**0.050**	18.5	**<0.001**
K	1	—	ns	—	ns	5.1	**0.025**	—	ns	—	ns	—	ns
Mg	1	—	ns	—	ns	—	ns	31.0	**<0.001**	10.1	**0.001**	—	ns
Ca	1	4.5	**0.034**	—	ns	—	ns	—	ns	—	ns	—	ns
Organic content	1	—	ns	5.6	**0.019**	7.4	**0.007**	5.1	**0.025**	7.3	**0.007**	—	ns
Anatomotype × soil moisture	4	19.7	**<0.001**	9.1	**<0.001**	1.3	0.265	0.6	0.639	6.1	**0.001**	14.0	**<0.001**
Anatomotype × *Alnus* sp.	4	6.7	**<0.001**	5.7	**0.001**	0.2	0.913	3.0	**0.019**	2.5	**0.047**	3.6	**0.001**
Anatomotype × site	4	4.0	**0.004**	1.0	0.423	1.3	0.279	2.9	**0.023**	4.5	**0.002**	4.4	**0.002**
Alnus sp. × soil moisture	1	10.2	**0.002**	9.4	**0.002**	0.6	0.441	0.5	0.495	7.2	**0.008**	11.1	**0.001**
Site × soil moisture	1	16.6	**<0.001**	6.3	**0.012**	0.1	0.867	1.4	0.245	12.2	**0.001**	22.4	**<0.001**
Alnus sp. × anatomotype × soil moisture	4	11.1	**<0.001**	10.9	**<0.001**	2.7	**0.049**	1.1	0.375	4.6	**0.004**	7.9	**<0.001**

Note: Nonsignificant interactions and covariates were successively removed from the analyses to provide more statistical power (*D*, ectomycorrhizal short-root diameter; *W*, ectomycorrhizal short-root tip dry mass; *L*, ectomycorrhizal short-root tip length; RTD, root tissue density; SRA, specific root area; SRL, specific root length; RTFW, ectomycorrhizal short-root-tip frequency per unit dry mass; RTFL, ectomycorrhizal short-root-tip frequency per unit length; ns, not significant).

both elongation of existing cells and formation of new cells within the apical meristem of a short root.

Two anatomotypes, *Alnicola* spp. and *T.* aff. *sublilacina*, dominated in both grey and black alder forests in Estonia. A further three anatomotypes in grey alder and four in black alder were more abundant than all other detected anatomotypes (Tedersoo et al. 2009).

We showed in our study that the functional parameters SRA and SRL incorporated the effects of all studied factors — EcM fungal partner, impact of tree species, and site and soil conditions: SRA and SRL differed significantly between anatomotypes, sites, and tree species. Of short-root morphological characteristics, *L*, *D*, and RTD independently characterize the size and inner structure of a root. At the same time, SRA and SRL, which are inversely dependent on *D* and RTD (Ostonen et al. 1999), are more intimately related to root function. However, SRA and SRL of short roots of *T.* aff. *sublilacina* and *L. cyathuliformis* s.l. were similar, despite significant differences between *D* and RTD for these anatomotypes. The mean *D* value and mean RTD may respond differently to different environmental conditions, and have opposite effects on SRL and SRA (Ryser 1998, 2006; Ostonen et al. 2007*b*). We found *D* to be strongly dependent on anatomotype and RTD on site. The strong dependency of RTD on site conditions suggests that it is a predictive functional indicator of mineral nutrition.

The inflow of N has been shown to be more efficient in *P. filamentosus* (Van der Heijden and Kuyper 2003). In our study, RTD was lowest for EcM roots colonized by *P. filamentosus*., suggesting more efficient mineral nutrient exchange than in roots colonized by dominant anatomotypes. A low RTD of roots characterized fast-growing grass species (Wahl and Ryser 2000), and we expect that a short root with a lower RTD can be considered more efficient because water and nutrient inflow would be facilitated. Based on SRA, SRL, and RTD, nutrient and water uptake should be most efficient in Porijõe, as RTD of EcM roots is low and SRA is high. A positive correlation between RTD and the proportion of cell walls in the stele has been reported for grasses (Wahl and Ryser 2000). The relationship between RTD variation in EcM short roots and the particular EcM fungal taxon needs further investigation.

The anatomotypes *Alnicola* spp. and *T.* aff. *sublilacina* were long and relatively thin at all seven sites, whereas *Pezizales* spp. formed short, stout root tips. Van der Heijden and Kuyper (2003) distinguished two ecological strategies for EcM short roots in *Salix repens*: a root-manipulation strategy based on elongation of roots, and root replacement. EcM fungi with a root-manipulation strategy strongly increased root length and their N economy was more effective than that of species with a root-replacement strategy. Our results suggest that *T.* aff. *sublilacina* and, particularly, *Alnicola* spp. follow the root-manipulation strategy, like the sister genus *Hebeloma* on *Salix* spp. (Van der Heijden and Kuyper 2003). Further, *Paxillus* spp. seem to use a root-replacement strategy. Root tips of *Paxillus* spp. were shorter, thicker, and more numerous per unit root length than those of *Alnicola* spp. EcM roots of *Paxillus* spp. had, on average, 1.5 times the number of root tips per unit root length than those of *Alnicola* spp.

Because of the diversity of the mycelial structures of EcM

Table 4. Morphological characteristics of short roots in EcM anatomotypes of *Alnus incana* and *A. glutinosa* stands.

Anatomotype	*D* (mm)		*L* (mm)		*W* (mg)		RTD ($kg \cdot m^{-3}$)		SRA ($m^2 \cdot kg^{-1}$)		SRL ($m \cdot g^{-1}$)		RTFW (no.·(mg dry mass)$^{-1}$)		RTFL (no.·cm^{-1})	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Alnus incana																
Alnicola spp.	0.338bc	0.007	**4.4**b	0.2	**0.047**ab	0.003	**116**a	4	**108**b	4	**106**b	6	**26.1**a	1.8	**2.5**a	0.1
Tomentella aff. *sublilacina*	0.334bc	0.006	**4.5**b	0.2	**0.057**ab	0.004	144b	3	85a	2	82a	4	**19.7**a	1.3	**2.4**a	0.1
Lactarius cyathuliformis s.l.	0.370c	0.028	4.0b	0.3	0.066bc	0.022	131ab	6	86ab	8	78ab	11	20.9a	3.1	2.6a	0.3
Tomentella aff. *ellisii*	**0.287**ab	0.016	5.4b	1.0	0.050ab	0.010	147ab	12	98ab	6	110ab	9	24.6a	5.4	2.3a	0.5
Genea–Humaria lineage sp.	0.384c	0.024	1.4a	0.3	0.024a	0.006	136ab	13	83ab	9	72ab	11	65.5b	18.8	8.6b	1.6
Alnus glutinosa																
Alnicola spp.	0.336x	0.004	**5.2**y	0.3	**0.062**y	0.003	**134**yz	3	**92**y	2	**89**z	3	**19.1**	0.1	**2.2**x	0.1
T. aff. *sublilacina*	0.351x	0.004	**5.3**y	0.2	**0.071**y	0.003	141z	2	83x	1	76xy	2	**15.6**	0.7	**2.0**x	0.1
L. cyathuliformis s.l.	0.389y	0.007	4.6y	0.4	0.067y	0.007	121xy	4	87xy	3	72xyz	3	17.3	1.4	2.4x	0.2
T. aff. *ellisii*	**0.372**xy	0.012	4.7y	0.4	0.060y	0.006	120xyz	8	95xy	7	83yz	8	18.2	1.7	2.3x	0.2
Tomentella sp. 1	0.439z	0.11	4.3y	0.5	0.080y	0.011	124xyz	8	77xy	4	56x	3	15.4	3.2	2.7x	0.3
Tomentella aff. *stuposa*	0.433z	0.016	4.2y	0.5	0.074y	0.010	122xyz	8	78xy	5	59xy	6	15.5	2.1	2.7x	0.3
Paxillus filamentosus	0.454z	0.011	2.9x	0.2	0.039x	0.002	93x	7	98xy	6	69xyz	5	25.6	1.5	3.7y	0.4

Note: Values followed by a different letter differ significantly (Tukey's test, $p < 0.05$) within *Alnus* species. Values in boldface type differ significantly between the two *Alnus* species (*D*, ectomycorrhizal (EcM) short-root diameter; *W*, EcM short-root-tip dry mass; *L*, EcM short-root-tip length; RTD, root tissue density; SRA, specific root area; SRL, specific root length; RTFW, EcM short-root-tip frequency per unit dry mass; RTFL, EcM short-root-tip frequency per unit length).

fungi, different exploration types could be distinguished, which are able to colonize the soil and organic substrates differently (Agerer 2001). In our study, two dominant anatomotypes — *Alnicola* spp. and *T.* aff. *sublilacina* — were of similar short-distance exploration types; however, the genus *Paxillus*, with its differentiated rhizomorphs, is characterized as a long-distance exploration type by Agerer (2001). Both *P. filamentosus* and *T.* aff. *stuposa* were exceptionally abundant in the middle-aged *A. glutinosa* stand at Sirgala, which is in an early-successional stage and has been planted on reclaimed opencast oil shale mining spoil. RDA revealed similarities between the Sirgala site and the studied grey alder forests, based on both short-root morphology and EcM fungal community structure. One explanation of the similarities in short-root morphology of the black alder plantation and grey alder stands at Sirgala could be that the habitat in Sirgala is drier than in the other black alder forests. On the other hand, the differences in short-root morphology and EcM fungal community structure could be related to lower N and P concentrations in developing soil. The external mycelium of *Paxillus involutus* contributed significantly to N and P nutrition of N- and P-deficient Norway spruce seedlings (Brandes et al. 1998). It has been proved that EcM fungi may differentially affect root length, relative N- and P-uptake efficiency (per unit root length) in *Salix* spp. For example, *Hebeloma* sp., with long EcM root tips, was 2–3 times less efficient in N uptake than *Paxillus* sp., with shorter EcM root tips (Van der Heijden and Kuyper 2003).

Our results indicate that both EcM fungi and the concentrations of macronutrients affected the size of root tips. For instance, the length of short root tips depended significantly on N, P, and K concentrations and organic-matter content in the soil, and diameter depended on N and Ca concentrations in the soil. The functional root traits RTD and SRA were significantly affected by the Mg concentration in the soil. Fertilizers, including N, P, K, Mg, and Ca, have been shown to affect fine-root morphology and biomass of hyphae in a *Pinus pinaster* stand (Bakker et al. 2009), and an increase in fine-root dry matter in Norway spruce seedlings in response to addition of Mg, N, P, and K was recorded (Zhang and George 2009). Further, the effects of macronutrients on short-root size could be mediated by EcM fungi. Species density (i.e., species richness per sample) and EcM colonization in our alder stands were significantly affected by soil K and Ca concentrations and organic-matter content (Tedersoo et al. 2009). Hence, our results confirm the existence of interrelations between short-root traits and EcM fungi. Nutrient fluxes within fungal hyphae are interdependent, i.e., a strong coupling of N, K, and Mg fluxes with long-distance P translocation in the mycorrhizal mycelium occurs (Jentschke et al. 2001)

The factors that favour the dominance of different anatomotypes are still unclear. However, it was demonstrated that EcM roots colonized by the most dominant anatomotype, *Alnicola* spp., had the highest SRA and SRL among all *Alnus* stands, which suggests a better cost to benefit ratio for EcM short roots in *Alnicola* spp.

The *A. glutinosa* trees at Sirgala and Songa originate from the same set of mother trees, and the significantly higher RTFL than in the other two stands could be one piece of evidence supporting the view that genetic variation within a

Table 5. Characteristics of ectomycorrhizal short roots of *Alnus incana* and *A. glutinosa* stands.

	D (mm)		*L* (mm)		RTD (kg·m^{-3})		SRA (m^2·kg^{-1})		SRL (m·g^{-1})		RTFL (no.·cm^{-1})	
Site	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Alnus incana												
Leidisoo	0.35b	0.01	4.3	0.3	138b	4	85a	4	79a	4	2.5	0.1
Saka	0.31a	0.01	4.2	0.2	135b	4	102b	4	111b	4	2.9	0.3
Porijõe	0.37b	0.01	3.8	0.3	112a	4	102b	4	89a	4	3.4	0.5
Alnus glutinosa												
Inka	0.37ab	0.01	5.7c	0.2	122a	3	95b	2	88b	3	2.0a	0.1
Songa	0.38c	0.01	4.5ab	0.2	137b	3	80a	2	69a	3	2.7b	0.1
Järvselja	0.34a	0.01	5.2bc	0.2	146b	3	81a	2	77ab	3	2.1a	0.1
Sirgala	0.37	0.01	4.1a	0.2	123a	3	90b	2	78ab	3	2.6b	0.1

Note: Values followed by a different letter differ significantly (Tukey's test, $p < 0.05$) within *Alnus* species. (*D*, ectomycorrhizal (EcM) short-root diameter; *W*, EcM short-root-tip dry mass; *L*, EcM short-root-tip length; RTD, root tissue density; SRA, specific root area; SRL, specific root length; RTFW, EcM short-roottip frequency per unit dry mass; RTFL, EcM short-roottip frequency per unit length.

tree species may have an important effect on root traits also. Our results are consistent with Korkama et al. (2006), who demonstrated a significant difference in root-tip frequency per fine-root (<2 mm) length between slow- and fast-growing clones of Norway spruce.

The results of our work show that to characterize the effects of different environmental factors in a forest ecosystem, a complex of EcM root traits should be analyzed. Reported changes in root morphology along ecological gradients (Godbold et al. 2003; Leuschner et al. 2004) raise the question of whether differences in environmental conditions cause changes in EcM community structure. Community structure and relative frequency/abundance of individual fungal species may change substantially in relation to topography and nutrient gradients (Lilleskov et al. 2002, 2008; Tedersoo et al. 2003, 2008; Toljander et al. 2006). No reports of consistent changes in EcM exploration types could be found in the literature, probably because single dominants may mask the overall shifts. Hence, a knowledge of EcM fungal community structure and the correlation of EcM fungus species with morphological and physiological patterns in short roots is important for understanding the relative roles of the functional adaptation of plants (root anatomy) and fungi (mantle thickness, abundance of external mycelium, species) to soil-quality and vegetation gradients.

Our data suggest that the particular species of EcM fungi drive EcM short-root traits, primarily independent variables such as diameter and length, and eventually functional parameters such as SRA or SRL of EcM roots in alders. The shifts in EcM communities may play an important role in morphological adaptation and mineral nutrition of plants. To elucidate the role played by EcM fungi in determining short-root functional properties and adaptation to different soil conditions, further investigation is needed.

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References

Abuzinadah, R.A., and Read, D.J. 1989. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. IV. The utilization of peptides by birch (*Betula pendula* L.) infected with different mycorrhizal fungi. New Phytol. **112**(1): 55–60. doi:10.1111/j.1469-8137.1989.tb00308.x.

Agerer, R. 1987–1998. Color atlas of ectomycorrhizae. 1st–11th eds. Einhorm-Verlag, Schwäbisch Gmünd.

Agerer, R. 1991. Characterization of ectomycorrhiza. *In* Techniques for the study of mycorrhiza. *Edited by* J.R. Norris, D.J. Read, and A.K. Varma. Academic Press, London, UK. pp. 25–73.

Agerer, R. 2001. Exploration types of ectomycorrhizae. Mycorrhiza, **11**(2): 107–114. doi:10.1007/s005720100108.

Baar, J., van Groenendael, J.M., and Roelofs, J.G.M. 2000. Are ectomycorrhizal fungi associated with *Alnus* of importance for forest development in wet environments? Plant Biol. **2**(5): 505–511. doi:10.1055/s-2000-7468.

Bakker, M.R., Jolicoeur, E., Trichet, P., Augusto, L., Plassard, C., Guinberteau, J., and Loustau, D. 2009. Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old *Pinus pinaster* stand. Tree Physiol. **29**(2): 229–238. doi:10.1093/treephys/tpn020. PMID:19203948.

Becerra, A., Pritsch, K., Arrigo, N., Palma, M., and Bartoloni, N. 2005. Ectomycorrhizal colonization of *Alnus acuminata* Kunth in northwestern Argentina in relation to season and soil parameters. Ann. For. Sci. **62**(4): 325–332. doi:10.1051/forest:2005027.

Brandes, B., Godbold, D.L., Kuhn, A.J., and Jentschke, G. 1998. Nitrogen and phosphorus acquisition by the mycelium of the ectomycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. New Phytol. **140**(4): 735–743. doi:10.1046/j.1469-8137.1998.00313.x.

Brundrett, M.C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. **154**(2): 275–304. doi:10.1046/j.1469-8137.2002.00397.x.

Brunner, I., and Horak, E. 1990. Mycoecological analysis of *Alnus* associated macrofungi in the region of the Swiss National Park as recorded by J. Favre (1960). Mycol. Helv. **4**: 111–139.

Brunner, I., Brunner, F., and Laursen, G.A. 1992. Characterization and comparison of macrofungal communities in an *Alnus tenuifolia* and an *Alnus crispa* forest in Alaska. Can. J. Bot. **70**(6): 1247–1258. doi:10.1139/b92-158.

Burgess, T., Malajczuk, N., and Grove, T.S. 1993. The ability of 16

ectomycorrhizal fungi to increase growth and phosphorus uptake by *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell. Plant Soil, **153**(2): 155–164. doi:10.1007/BF00012988.

Chalot, M., Javelle, A., Blaudez, D., Lambilliote, R., Cooke, R., Sentenac, H., Wipf, D., and Botton, B. 2002. An update on nutrient transport processes in ectomycorrhizas. Plant Soil, **244**(1–2): 165–175. doi:10.1023/A:1020240709543.

Courty, P.E., Pritsch, K., Schloter, M., Hartmann, A., and Garbaye, J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytol. **167**(1): 309–319. doi:10.1111/j.1469-8137.2005.01401.x. PMID: 15948852.

Dilly, O., Bach, H.-J., Buscot, F., Eschenbach, C., Kutsch, W.L., Middelhoff, U., Pritsch, K., and Munch, J.C. 2000. Characteristics and energetic strategies of the rhizosphere in ecosystems of the Bornhöved Lake district. Appl. Soil Ecol. **15**(2): 201–210. doi:10.1016/S0929-1393(00)00096-2.

FAO-UNESCO. 1994. Soil map of the world. Revised legend. ISRIC, Wageningen, Netherlands.

Fitter, A.H. 1991. Characteristics and functions of root systems. *In* Plant roots — the hidden half. *Edited by* Y. Waisel, A. Eshel, and U. Kafkafi. Marcel Dekker, New York. pp. 3–24.

Godbold, D.L. 2005. Ectomycorrhizal community structure: linking biodiversity to function. Prog. Bot. **66**: 374–391. doi:10.1007/3-540-27043-4_15.

Godbold, D.L., Fritz, H.-W., Jentschke, G., Meesenburg, H., and Rademacher, P. 2003. Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. Tree Physiol. **23**(13): 915–921. PMID:14532015.

Horton, T.R., and Bruns, T.D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol. Ecol. **10**(8): 1855–1871. doi:10.1046/j.0962-1083.2001.01333.x. PMID:11555231.

Jentschke, G., and Godbold, D.L. 2000. Metal toxicity and ectomycorrhizas. Physiol. Plant. **109**(2): 107–116. doi:10.1034/j.1399-3054.2000.100201.x.

Jentschke, G., Brandes, B., Kuhn, A.J., Schröder, W.H., and Godbold, D.L. 2001. Interdependence of phosphorus, nitrogen, potassium and magnesium translocation by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol. **149**(2): 327–337. doi:10.1046/j.1469-8137.2001.00014.x.

Korkama, T., Pakkanen, A., and Pennanen, T. 2006. Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. New Phytol. **171**(4): 815–824. doi:10.1111/j.1469-8137.2006.01786.x. PMID:16918552.

Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W., and van Breemen, N. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol. Evol. **16**(5): 248–254. doi:10.1016/S0169-5347(01)02122-X. PMID: 11301154.

Leuschner, C., Hertel, D., Schmid, I., Koch, O., Muhs, A., and Hölscher, D. 2004. Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil, **258**(1): 43–56. doi:10.1023/B:PLSO.0000016508.20173.80.

Lilleskov, E.A., Fahey, T.J., Horton, T.R., and Lovett, G.M. 2002. Belowground ectomycorrhizal community change over a nitrogen deposition gradient in Alaska. Ecology, **83**: 104–115. doi:10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2.

Lilleskov, E.A., Wargo, P.M., Vogt, K.A., and Vogt, D.J. 2008. Mycorrhizal fungal community relationship to root nitrogen concentration over a regional atmospheric nitrogen deposition gradient in the northeastern USA. Can. J. For. Res. **38**(5): 1260–1266. doi:10.1139/X07-211.

Lõhmus, K., Truu, M., Truu, J., Ostonen, I., Kaar, E., Vares, A., Uri, V., Alama, S., and Kanal, A. 2006. Functional diversity of culturable bacterial communities in the rhizosphere in relation to fine-root and soil parameters in alder stands on forest, abandoned agricultural, and oil-shale mining areas. Plant Soil, **283**(1–2): 1–10. doi:10.1007/s11104-005-2509-8.

Mander, Ü., Lõhmus, K., Teiter, S., Uri, V., and Augustin, J. 2008. Gaseous nitrogen and carbon fluxes in riparian alder stands. Boreal Environ. Res. **13**: 231–241.

Ostonen, I., and Lõhmus, K. 2003. Proportion of fungal mantle, cortex and stele of ectomycorrhizas in *Picea abies* (L.) Karst. in different soils and site conditions. Plant Soil, **257**(2): 435–442. doi:10.1023/A:1027305906159.

Ostonen, I., Lõhmus, K., and Lasn, R. 1999. The role of soil conditions in fine root ecomorphology in Norway spruce (*Picea abies* (L.) Karst.). Plant Soil, **208**(2): 283–292. doi:10.1023/A:1004552907597.

Ostonen, I., Lõhmus, K., Alama, S., Truu, J., Kaar, E., Vares, A., Uri, V., and Kurvits, V. 2006. Morphological adaptations of fine roots in scots pine (*Pinus sylvestris* L.), silver birch (*Betula pendula* roth.) and black alder (*Alnus glutinosa* (L.) Gaertn.) stands in recultivated areas of oil shale mining and semicoke hills. Oil Shale, **23**: 187–202.

Ostonen, I., Lõhmus, K., Helmisaari, H.-S., Truu, J., and Meel, S. 2007*a*. Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. Tree Physiol. **27**(11): 1627–1634. PMID:17669752.

Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, A., Pronk, A., Vanguelova, E., Weih, M., and Brunner, I. 2007*b*. Specific root length as an indicator of environmental change. Plant Biosyst. **141**: 426–442. doi:10.1080/11263500701626069.

Paal, J. 1997. Classification of Estonian vegetation site types. Ministry of Environment, Tallinn, Estonia. [In Estonian with English summary.]

Perez-Moreno, J., and Read, D.J. 2000. Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. **145**(2): 301–309. doi:10.1046/j.1469-8137.2000.00569.x.

Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., and Hendrick, R.L. 2002. Fine root architecture of nine North American trees. Ecol. Monogr. **72**: 293–309. doi:10.1890/0012-615(2002)072[0293:FRAONN]2.0.CO;2.

Pritsch, K., Boyle, H., Munch, J.C., and Buscot, F. 1997*a*. Characterization and identification of black alder ectomycorrhizas by PCR/RFLP analyses of the rDNA internal transcribed spacer. New Phytol. **137**(2): 357–369. doi:10.1046/j.1469-8137.1997.00806.x.

Pritsch, K., Munch, J.C., and Buscot, F. 1997*b*. Morphological and anatomical characterisation of black alder *Alnus glutinosa* (L.) Gaertn. ectomycorrhizas. Mycorrhiza, **7**(4): 201–216. doi:10.1007/s005720050182.

Rooma, I., and Paal, J. 2001. Soils of the Estonian Klint forests. Year-book of the Estonian Naturalists' Society, **80**: 178–209.

Ryser, P. 1998. Intra- and interspecific variation in root length, root turnover and the underlying parameters. *In* Inherent variation in plant growth: physiological mechanisms and ecological consequences. *Edited by* H. Lambers, H. Poorter, and M.M.I. Van-Vuuren. Backhuys Publishers, Leiden, Netherlands. pp. 441–465.

Ryser, P. 2006. The mysterious root length. Plant Soil, **286**(1–2): 1–6. doi:10.1007/s11104-006-9096-1.

Tedersoo, L., Kõljalg, U., Hallenberg, N., and Larsson, K.-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across

substrate layers including coarse woody debris in a mixed forest. New Phytol. **159**(1): 153–165. doi:10.1046/j.1469-8137.2003.00792.x.

Tedersoo, L., Suvi, T., Jairus, T., and Kõljalg, U. 2008. Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. Environ. Microbiol. **10**: 1189–1201. doi:10.1111/j.1462-2920.2007.01535.x. PMID:18266759.

Tedersoo, L., Suvi, T., Jairus, T., Ostonen, I., and Põlme, S. 2009. Revisiting ectomycorrhizal fungi of the genus *Alnus*: differential host specificity, diversity and determinants of the fungal community. New Phytol. **182**(3): 727–735. doi:10.1111/j.1469-8137.2009.02792.x. PMID:19320837.

ter Braak, C.J.F., and Šmilauer, P. 2002. Reference manual and CanoDraw for Windows: user's guide: software for canonical ordination. Version 4.5. Microcomputer Power, Ithaca, N.Y.

Toljander, J.F., Eberhardt, U., Toljander, Y.K., Paul, L.R., and Taylor, A.F.S. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytol. **170**(4): 873–884. doi:10.1111/j.1469-8137.2006.01718.x. PMID:16684245.

Van der Heijden, E.W., and Kuyper, T.W. 2003. Ecological strategies of ectomycorrhizal fungi of *Salix repens*: root manipulation versus root replacement. Oikos, **103**(3): 668–680. doi:10.1034/j.1600-0706.2003.10638.x.

Vares, A., Lõhmus, K., Truu, M., Truu, J., Tullus, H., and Kanal, A. 2004. Productivity of black alder (*Alnus glutinosa* (L.) Gaertn.) plantations established on reclaimed oil shale mining spoil and mineral soils in relation to rhizosphere conditions. Oil Shale, **21**: 47–62.

Wahl, S., and Ryser, P. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytol. **148**(3): 459–471. doi:10.1046/j.1469-8137.2000.00775.x.

Withington, J.M., Reich, P.B., Oleksyn, J., and Eissenstat, D.M. 2006. Comparison of structure and life span in roots and leaves among temperate trees. Ecol. Monogr. **76**(3): 381–397. doi:10.1890/0012-9615(2006)076[0381:COSALS]2.0.CO;2.

Zhang, J.L., and George, E. 2009. Root proliferation of Norway spruce and Scots pine in response to local magnesium supply in soil. Tree Physiol. **29**(2): 199–206. doi:10.1093/treephys/tpn016. PMID:19203945.