





**KATRIN ROSENVOLD**

Factors affecting  
ectomycorrhizal roots  
and rhizosphere in silver birch stands



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## LIST OF ORIGINAL PUBLICATIONS

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- III. Kuznetsova T, Rosenvald K, Ostonen I, Helmisaari H-S, Mandre M, Lõhmus K (2010) Survival of black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) seedlings in a reclaimed oil shale mining area. *Ecological Engineering*, 36(4), 495–502
- IV. Lõhmus K, Kull A, Truu J, Truu M, Kaar E, Ostonen I, Meel S, Kuznetsova T, Rosenvald K, Uri V, Kurvits V, Mander Ü (2007) The reclamation of the North Estonian oil shale mining area. Mander Ü, Wiggering H, Helming K (ed.). *Multifunctional land use: Meeting future demands for landscape goods and services* (387–401). Berlin Heidelberg: Springer
- V. Rosenvald K, Ostonen I, Uri V, Varik M, Tedersoo L, Lõhmus K. Tree age effect on fine root and leaf morphology in a silver birch forest chronosequence. (Manuscript)

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### Author's contribution to each paper

	I	II	III	IV	V
Original idea	*	*			*
Study design	*	*			*
Data collection	*	*	*	*	*
Data analyses	*	*	*	*	*
Manuscript preparation	*	*	*	*	*

## ABSTRACT

Limited nutrient acquisition from soil is a key process limiting productivity in boreal forest, and nutrition strategies change during stand development. The overall objective of the doctoral thesis was (i) to find and quantify the response of EcM root morphology and rhizosphere effect of silver birch (*Betula pendula*) to different site conditions, (ii) to ascertain tree-age-related changes in EcM root morphology and rhizosphere effect in relation to nutrient status of silver birch.

EcM morphology and rhizosphere effect were studied in silver birch stands for three forest categories according to previous land use: natural forest, forest on previous agricultural land and in reclaimed opencast oil shale mine area. The response of EcM morphology and rhizosphere effect to site conditions was studied in seven young silver birch stands (8–14 yrs) growing on abandoned agricultural land. Age-related changes in EcM morphology and rhizosphere effect were studied in two birch stand chronosequences growing in natural *Oxalis* site type forest (6, 14, 32, 45, 60 yrs old) and in reclaimed mine area (1, 2, 3, 5, 29, 40 yrs old). EcM morphology and rhizosphere effect of silver birch were compared with black alder (*Alnus glutinosa*) in plantations growing in reclaimed oil shale mining area. Short-root morphological parameters were measured using WinRHIZO™ Pro. Summed activity (SA) of bacteria (by BIOLOG Ecoplate™) was determined for rhizosphere (R) and bulk soil (S) to reveal the extent of the rhizosphere effect. Tree nutrient (NPK) status was expressed by leaf nutrient concentrations.

EcM morphology and rhizosphere effect were influenced by site properties especially by previous land use as well as tree age. The ontogenetic changes in EcM root morphology occurred irrespective of site properties – EcM root morphological parameters had a similar general pattern in natural forest and reclaimed oil shale mine area chronosequence despite the very different site conditions. Age-related changes in EcM root morphology occurred faster in younger age, before the age of 5–10 years. The functional balance between physiology and morphology of leaves and roots, as functionally homogeneous and most active parts of trees, appeared during stand development in natural forest. The specific leaf area (SLA) and specific EcM root area (SRA) as well as leaf N decreased simultaneously with tree age, probably as a result of down-regulation of growth. Age-related decrease of silver birch SRL and SRA was caused both by increasing EcM root diameter and tissue density. High EcM root SRL and SRA can be considered as root N nutrition stress indicators for silver birch – both increased in case of low leaf N%.

To find the possible effect of exploration types of colonising EcM species on root morphology, the dominating EcM fungal species and their exploration types were determined in a pilot study for age classes 6, 14, and 32. Molecular analyses revealed 33 EcM fungal species among the dominant morphotypes in three studied age classes. However, the distribution of the four exploration types (contact, short-, medium-, long-distance) of dominating EcM was similar across

the chronosequence. The dominating EcM root tip colonisers belonged most frequently to the short or medium exploration type. The exploration type of EcM fungi significantly affected EcM root morphology.

The rhizosphere effect was higher by lower pH and higher root N% on agricultural land. Rhizosphere processes in silver birch stands contribute to soil pH decrease in mine area with alkaline soil, and increase soil pH in natural forest with acidic soil to improve tree mineral nutrition. In young birch forests on agricultural land, rhizosphere acidification occurred in the studied stands when bulk soil pH(KCl) > 5. The impact of tree species was significant; compared to black alder, silver birch was oriented more towards fine-root adaptations than support of rhizosphere communities.

Strategies improving root nutrition – morphological adaptation and rhizosphere effect – depended on different environmental factors, which means they are based on different regulatory mechanisms. The results of the thesis provide cognitive basis for formulating new hypotheses concerning fine root and rhizosphere processes under changing environmental conditions.



## LIST OF ABBREVIATIONS

A	–	age
agric.	–	agricultural
CLPP	–	community level physiological profiles
D	–	diameter
DBH	–	diameter at breast height
EcM	–	ectomycorrhizal, ectomycorrhiza
K	–	potassium
L	–	length
N	–	nitrogen
NPP	–	net primary production
M	–	mass
P	–	phosphorus
R	–	rhizosphere
RTD	–	root tissue density
RTF <sub>M</sub>	–	root tip frequency per 1mg root mass
S	–	bulk soil
SE	–	standard error
SA	–	summed bacterial functional activity
SLA	–	specific leaf area
SRA	–	specific root area
SRL	–	specific root length
yr	–	year

# I. INTRODUCTION

## I.1. Background

The acquisition of nutrients from the soil is a key process limiting productivity and carbon sequestration in forest ecosystems. A tree root system consists of coarse and fine branches – the coarse roots and the fine roots. Coarse roots have undergone secondary thickening and have a woody structure; their main function is transport and anchoring. Fine roots (generally considered roots with  $D < 2$  mm) have also been regarded as short-lived and recognised as the most important component contributing to below-ground carbon fluxes in forest ecosystems, accounting for up to 75% of the annual net primary production (Vogt 1991). According to a recent review by Finér et al. 2011, the mean fine root biomass is  $526 \pm 321$  in boreal forest and  $775 \pm 474$  g m<sup>-2</sup> in temperate forest.

The fine root system of dominating boreal trees is heterorhizic, consisting of long, mainly woody roots and short roots (Sutton 1980). The woody roots may have limited capacity for nutrient uptake (Addams 1946), but their transport function is more important. The short roots have predominantly primary structure, a living cortex, and usually mycorrhizal association to acquire water and nutrients (Brundrett 2002; Smith and Read 2008). Nutrient uptake for dominant boreal tree species including silver birch (*Betula pendula* Roth.) occurs mainly through colonising EcM fungi. Mantles of EcM fungi cover nearly all nutrient absorbing regions in roots (Taylor and Peterson 2005), and their external mycelium extends out to the soil, exploiting huge soil volumes for nutrient uptake. Nutrient transfer between the plant and fungus occurs bidirectionally – the soil-derived nutrients are exchanged for carbohydrates from the host plant. EcM fungi increase the uptake rate of nutrients by increasing physical access to soil, changing the soil chemistry by enzymatic activity, and altering the bacterial community of the mycorrhizosphere (Simard et al. 2002). Recent investigations have proved that the colonising EcM fungi have a significant impact on root tip morphology (Comas and Eissenstat 2009; Ostonen et al. 2009; Sun et al. 2010).

Different fine-root size-classes within  $< 2$  mm do not function in the same way (Pregitzer et al. 2002; Hishi 2007). Since EcM roots are functionally homogeneous and exhibit particularly high physiological activity due to their dominance in total root length and surface area, it is better to use the EcM root compartment in investigations of root structure and functioning instead of fine roots.

Nutrient uptake by roots is mediated by processes taking place in the rhizosphere, where in comparison with the bulk soil the biomass and activity of microorganisms is enhanced by root exudation and other fluxes of rhizodeposition (Lynch and Whipps 1990; Morgan et al. 2005; Berg and Smalla 2009; Jones et al. 2009). The term rhizosphere was introduced by Lorenz Hiltner in 1904 to describe the portion of soil where microorganism-mediated processes are under the influence of the root system.

From less than 10% up to 44% of the photosynthetically fixed carbon is excreted by the root (Grayston et al. 1996; Bais et al. 2006).

As roots play a crucial role in nitrogen (N) uptake, it has been suggested that root N concentration reflects the N supply in the same way as foliar N concentration (Adams et al. 1987). However, there have been few attempts that have reported N concentrations of foliage, roots and EcM tips or mycelia from the same sites (Helmisaari et al. 2009). The functional balance between leaves and roots can be described in terms of mass allocation and morphology or alternatively in terms of leaf and root “activity” (Wright and Westoby 2000).

Soil resources are often poorly available to organisms due to the capacity of soil matrix to bind water and nutrients, so that roots have to adapt – to influence their immediate environment and to optimize their functional architecture to explore and make use of resources in heterogeneous soils (Hinsinger et al. 2009).

## **1.2. Fine-root and leaf morphological adaptations**

Morphological adaptations of fine roots affect the capacity of roots to acquire nutrients from soil (Hodge et al. 2009), and root morphology plays an important role in balancing costs and benefits of root growth and activity. The functional characteristics of absorbing roots – specific root length (SRL) and specific root area (SRA) are both affecting root efficiency because resource acquisition is proportional to length (especially for low mobility nutrients with low concentration in soil) or surface area, and root (construction and maintenance) cost is proportional to mass (Eissenstat and Yanai 1997; Lõhmus et al. 1989; Ostonen et al. 2007a; Richardson et al. 2009). SRL and SRA are both functions of root diameter (D) and root tissue density (RTD):

$$\text{SRL} = 4/(\pi D^2 \text{RTD}),$$
$$\text{SRA} = 4/(D \text{RTD}).$$

The effect of environmental conditions on fine-root morphology of deciduous tree species has been analysed in a number of studies (e.g. Ostonen et al. 2007ab; Leuschner et al. 2009; Richter et al. 2007; Makita et al. 2011). However, only few of them deal with functionally homogeneous EcM or short root compartment (Ostonen et al. 1999, 2007b, 2009; Helmisaari et al. 2009), although these fine-root segments can indicate the root responses to soil nutrients better. Moreover, morphological parameters of EcM roots reflect different fine-root adaptation and acclimation strategies for ectomycorrhizal trees because EcM roots are a short-lived and continuously renewable functional compartment of the fine-root system (Pregitzer et al. 2002; Guo et al. 2004; Ostonen et al. 2007b).

The influence of forest ageing on fine-root morphology and the functional role of root adaptations and acclimations is poorly studied; in recent years, a few papers have been published (Löhmus et al. 2006b; Børja et al. 2008; Jagodziński and Kałucka 2010). Stand-age-related variation in fine and EcM root morphology is important for understanding complex ecophysiological changes during forest succession and for mitigating management impacts on forest 'ecosystem function' (Thomas 2010).

Finest root orders, including EcM roots, and leaves are similar in terms of resource acquisition and being ephemeral (Wells and Eissenstat 2001; Pregitzer et al. 2002); leaves transfer carbon from the atmosphere for tree growth and have other metabolic functions through photosynthesis. The ability of trees to exhibit plasticity in leaf morphology, allowing them to optimise photosynthetic efficiency as well as other ecophysiological functions is well known. Specific leaf area (SLA) describes the transpiring and photosynthesizing surface area that can be constructed from a unit dry mass of organic matter. The specific leaf area (SLA) is a key trait in plant growth, and it has been used as an indicator of the potential for light-resource utilization and hence plant photosynthetic capacity (Lambers et al. 2006). Tree-age-related changes in leaf morphology of deciduous species are less studied than those of coniferous species. All reviewed papers about deciduous species report the decrease of SLA with increasing tree age (Mediavilla and Escudero 2009) and/or tree size (Sellin and Kupper 2006; Niklas and Cobb 2008, 2010; Thomas 2010). Hence, the morphology of EcM roots and leaves affects their functions and changes with tree age. There must be coordination between aboveground and belowground parts with respect to the acquisition and allocation of limiting resources and adaptation to different stressors (Wright and Westoby 2001; Liu et al. 2010). Relations between traits of functionally homogeneous roots and leaves have been studied by Withington 2006, who found a correlation between SLA and SRL (of first and second order roots only) when 11 tree species were included; the relation was insignificant if only *Pinacea* were included. Reich et al. 1998 studied seedlings of nine tree species, and showed a high correlation between SLA and SRL (of whole root system) across all the species. However, the present knowledge about interaction between leaf and fine root or EcM root morphology within a tree species is still limited.

### **1.3. Rhizosphere effect**

In addition to the morphological adaptation of roots, using rhizodeposition to support root-associated microorganisms is another strategy of trees to improve mineral nutrition (Löhmus et al. 2006a). Due to rhizodeposition (lysates, exudates, secretions, mycilage) microbial processes which support mineral nutrition, such as mineralisation (Phillips and Fahey 2006; Bader and Cheng 2007; Mueller and Shann 2007; Zhao et al. 2007), and enzymatic activities, e.g. acid

phosphatase activity (Radersma and Grierson 2004; Liu et al. 2006) are usually higher in the rhizosphere compared to bulk soil. The phenomenon is well-known as rhizosphere effect, which is considered to be the influence exerted by the growing plant on its immediate environment – in the rhizosphere (Lochhead and Rouatt 1955). Rhizosphere effect can be measured as the ratio or the difference between rhizosphere and bulk soil parameters. If the ratio or the difference is higher, microbial processes in the rhizosphere are more promoted and the rhizosphere effect is higher, and thus, nutrient uptake by roots should be improved respectively (Richardson et al. 2009).

Rhizosphere effect depends highly on soil nutrient status and other soil properties (Berg and Smalla 2009; Jones 2009). The mechanisms of age-related shifts in rhizosphere effect are not fully understood and need further investigation. In forest ecosystems, they are probably caused by physiological and structural changes during tree ontogenesis but also by altered biotic and abiotic site conditions (e.g. soil properties including shifts in microbial communities, canopy composition, tree density, etc) in stand development. The contribution of ectomycorrhizal fungi by mineral weathering and tree nutrition processes is well known and proved, but the role of rhizosphere bacterial communities in root nutrition remains poorly documented (Courty et al. 2010).

#### **I.4. Silver birch**

Fine-root morphology and rhizosphere effect have been less thoroughly investigated for trees than for herbaceous plants. Among different tree species, more attention has been paid to coniferous than to deciduous tree species. The main species studied in the doctoral thesis was silver birch (I–V). Silver birch together with downy birch (*B. pubescens* Ehrh.) are commercially the most important broadleaved tree species in Northern Europe (Hynynen et al. 2010), and birch stands comprise 31% of forests in Estonia. Silver birch is a fast-growing pioneer species, which due to large seed fall and high seed dispersal readily colonises open ground. A dense rooting, high fine-root biomass and foraging capacity (Priha et al. 1999; Curt and Prevosto 2003) as well as fast growth make silver birches competitive as a pioneer tree species in fertile sites (Perala and Alm 1990; Hynynen et al. 2010). Birch roots adapt to difficult conditions like stony mining spoil – roots proliferate in the upper soil, but sinkers penetrate deep soil layers (Perala and Alm 1990). Fine-root morphology and/or rhizosphere effect of silver birch was compared with black alder (*Alnus glutinosa* (L.) Gaertn.) in papers III and IV, which belongs to the same family (*Betulaceae*) as silver birch, and also with a coniferous tree species, Scots pine (*Pinus sylvestris* L.) in paper III.

## **1.5. Objectives**

The overall objective of the doctoral thesis was (i) to quantify the response of EcM root morphology and rhizosphere effect to different site conditions, (ii) to ascertain tree-age-related changes in EcM root morphology and rhizosphere effect in relation to tree nutrient status. The specific aims of this research were:

- 1) to quantify the variation in silver birch EcM root morphological parameters at different forest categories according to previous land use and to assess the sensitivity of root parameters to site conditions;
- 2) to find whether and how NPK nutrient status (assessed as leaf NPK%) and root N concentration of silver birch are related to EcM root morphological parameters and rhizosphere effect;
- 3) to find the influence of site conditions on ontogenetic changes in EcM root morphology by comparing the dynamics of morphological parameters of EcM roots of silver birch in two chronosequences growing in different ecosystems: *Oxalis* type forest and reclaimed oil shale mining area;
- 4) to estimate the response of rhizosphere effect to the increasing stand age and improved nutrient supply in a birch stand chronosequence in reclaimed oil shale mining area;
- 5) to analyse the influence of soil characteristics on rhizosphere effect of silver birch;
- 6) to ascertain the possible interactions between EcM root morphology and rhizosphere effect for silver birch and black alder;
- 7) to carry out a pilot study of the dominating EcM colonisers in root tips of silver birch in a natural forest chronosequence to analyse their impact on EcM root morphology;
- 8) to compare the dynamics of mean specific areas of EcM roots as well as leaves in the silver birch natural forest chronosequence;
- 9) to find and analyse between-species (silver birch, black alder, Scots pine) differences in EcM root morphology and rhizosphere effect.

## **1.6. Hypotheses**

1. EcM root morphology and rhizosphere effect of silver birch are related to nutrient status of trees reflecting site fertility.
2. Previous land use and tree age affect EcM root morphology of silver birch.
3. Age-trends of EcM root morphological parameters are different for natural forest and for forest in reclaimed oil shale mining area.
4. Changes in silver birch EcM root morphology occur faster in young trees.
5. EcM root and leaf traits during silver birch stand development are coordinated.
6. EcM root and rhizosphere adaptation strategies differ between tree species.
7. EcM root morphology of silver birch is influenced by exploration type of fungal taxa involved.

## 2. MATERIAL AND METHODS

### 2.1. Description of study sites

EcM morphology and rhizosphere effect were studied in silver birch stands for three forest categories according to previous land use: natural forest (V), forest on previous agricultural land (I) and in reclaimed opencast oil shale mine area (II, III, IV). The response of EcM morphology and rhizosphere effect to site conditions was studied in seven young silver birch stands (8–14 yrs) at the same development stage growing on abandoned agricultural land; these stands were selected to represent a broad soil pH gradient (Table 1) because among soil parameters, soil pH has the largest effect on the diversity and richness of soil bacterial communities (Fierer and Jackson 2006). The young birch sites were located throughout Estonia to ensure a wide spectrum of habitats with different abiotic and biotic factors. Temporal dynamics of EcM morphology and rhizosphere effect was studied in two birch chronosequences – in natural forest and in reclaimed mine area. The stands of the forest chronosequence regenerated naturally after clear-cut and belong to the *Oxalis* forest site type characterized by fertile and acidic soils (Table 1; Table 1 in V). The second birch stand chronosequence growing in reclaimed oil shale mining area can be considered as an

**Table 1.** Soil characteristics of studied stands. The age of stands on previous agricultural land varied between 8 and 14 years.

Forest category	Stand age/ name	Soil		
		Type	N%	pH
Natural forest	3	Umbric Gleysol	0.871	3.0
	6			
	14	Umbrisol	0.253	3.7
	32	Endogleyic Umbrisol	0.226	3.9
	45	Albeluvisol	0.117	3.9
	60	Umbrisol	0.157	3.8
Reclaimed mine area	1	Mining spoil	0.031	8.1
	2		0.023	7.9
	3		0.039	7.5
	5	0.050	7.4	
	29	Calcaric Regosol	0.325	6.9
	40	Calcaric Regosol	0.329	7.0
Previous agricultural land	Kassi	Haplic Planosol	0.116	3.8
	Väljaküla	Stagnic Luvisol	0.091	4.9
	Lutsu	Stagnic Luvisol	0.074	5.2
	Kambja	Haplic Albeluvisol	0.092	5.2
	Haaslava	Haplic Cambisol	0.117	5.7
	Pühatu	Haplic Cambisol	0.157	6.9
	Reigi	Rendzic Leptosol	0.258	7.0

example of the primary succession of forest ecosystem on stony and alkaline mining refuse which is accelerated by planting trees (Table 1; Table 2 in II). EcM morphology and rhizosphere effect of silver birch were compared with black alder (*Alnus glutinosa*) and Scots pine (*Pinus sylvestris*) in plantations growing in reclaimed oil shale mining area (III, IV).

## 2.2. Soil, root, and leaf sampling

Soil and root samples were collected in October 2004–2006 (II), 2005 (III), 2006 (I), 2008–2009 (V). Ten initial samples were randomly taken from 0 to 10 cm soil layer in stands growing in reclaimed oil shale mining area and from 0 to 20 cm soil layer in other sites. A random fine-root ( $d < 2$  mm) subsample was taken from each initial sample for morphological analysis. The remaining soil and roots from the samples of a stand were bulked to get a composite sample for microbiological and chemical analysis. A composite sample was formed and processed according to Gobran and Clegg (1996). Roots were carefully removed by hand from the field-moist mineral soil. Both the dead and coarse roots ( $\geq 2$  mm in diameter) as well as black alder nodules were excluded. Living roots were differentiated from dead roots using visible criteria: resilience, texture, colour of bark and xylem (Vogt and Persson, 1991). The live fine roots were carefully shaken manually for 1 min. in a plastic container to separate the soil aggregates from the roots. The fine roots with adhering soil gave the rhizosphere fraction. Mineral soil was passed through a 2 mm mesh sieve to give the bulk soil fraction.

Leaf samples for determining NPK concentration were taken from the tree crown sections (vertical layers) of model trees in August 2002–2004 (I, Uri et al. 2007ab) and 2008–2009 (V). The concentrations of leaf NPK and SLA of model trees were calculated as the weighted averages, considering the share of a particular section in the tree leaf biomass.

## 2.3. Estimation of morphological parameters of EcM roots and leaves

Prior to measuring morphological parameters, the roots of ten samples per stand were washed with tap water to remove the soil particles. Two random EcM root subsamples (about 20 living root tips per subsample) were taken per sample, near 400 tips per stand. All studied root tips were, according to macroscopic features, ectomycorrhizal.

EcM root length, projection area, and mean diameter of a subsample were measured using WinRHIZO™ Pro 2003b software. After measuring, EcM root samples were dried at 70°C until constant mass and weighed to 0.01mg. The method for determining EcM root morphological parameters: mean EcM root



length (L; mm), specific root area (SRA;  $\text{m}^2 \text{kg}^{-1}$ ), specific root length (SRL;  $\text{m g}^{-1}$ ), root tissue density (RTD;  $\text{kg m}^{-3}$ ), and root tip frequency per 1 mg dry mass ( $\text{RTF}_M$ ;  $\text{mg}^{-1}$ ) is given in detail in Ostonen et al. 1999 and 2007b.

Sampled leaves were dried under pressure and each single leaf area (including the petiole) was measured using the program WinFOLIA (Regent Instruments, Inc.). Measured leaves were dried until constant mass; each leaf was weighed to 0.1 mg, and specific leaf area (SLA) was calculated.

## **2.4. Identification of dominating EcM morphotypes**

For 6-, 14-, and 32-year-old stands of natural forest chronosequence, dominant morphotypes were identified from the washed root samples, and morphological parameters of EcM roots were measured by morphotype. The root tips from three additional fine-root fragments (5–7 cm in length) of each root sample were sorted into morphotypes on the basis of the colour and texture of the fungal mantle, hyphae and rhizomorphs. Three to five dominating morphotypes were identified, and their relative proportions of root tips were scored. A morphotype was counted as dominant when its relative abundance exceeded 20% of all tips on the root fragments in a subsample. Random EcM root subsamples for morphological studies were taken from each morphotype sample. For species-level identification of root-colonising EcM, molecular analysis was applied for three individual root tips of each morphotype per sample (more detailed description is given in paper V). Identified morphotypes were divided into four exploration types: contact, short-, medium-, and long-distance according to Agerer (2001).

## **2.5. Chemical analyses**

Soil nitrogen was determined according to Kjeldahl method, and soil available (ammonium lactate extractable) phosphorus was performed by flow injection analysis. Loss on ignition of soil was determined at 360 °C. Leaf nitrogen concentration was determined by block digestion and steam distillation methods. Leaf phosphorus concentration was measured as Kjeldahl phosphorus, and leaf potassium content was determined by flame photometry. Analyses were performed at the Laboratory of Biochemistry of the Estonian University of Life Sciences. The total N concentrations in EcM roots were determined using CHN analyser Perkin-Elmer 6400 in the laboratory of the Department of Geology of the University of Tartu.

## 2.6. Microbiological methods

We measured the rhizosphere effect on summed bacterial functional activity and community diversity as rhizosphere/soil ratios and on pH as the pH difference between rhizosphere and bulk soil (I, II). Summed bacterial functional activity of rhizosphere ( $SA_R$ ) and bulk soil ( $SA_S$ ) were determined by community level physiological profiles (CLPP) technique using the Biolog EcoPlate (Biolog Inc.) method (I, II). The CLPP method assesses the fast-growing cultivable part of the microbial community, which partly reflects the changes in total microbial community in rhizosphere (Söderberg et al. 2004). This technique has been proved to have discriminative power especially in the case of contrasting conditions and wide soil pH gradient (Löhmus et al. 2006a). Biolog Ecoplates contain substrates that are known to be plant root exudates. Higher activity ratio  $SA_R/SA_S$  is considered to indicate higher rhizosphere effect and greater support to the rhizosphere microbial communities (Löhmus et al. 2006a). Metabolic diversity of the bulk soil ( $MD_S$ ) and rhizosphere ( $MD_R$ ) was measured as Shannon diversity indices; the metabolic diversity ratio  $MD_R/MD_S$  was computed (I).

Bacterial community diversity was measured using DNA-based denaturing gradient gel electrophoresis (DGGE) technique (I, II). The similarity in bacterial community between rhizosphere and bulk soil was expressed as Pearson  $r$  – the coefficient of correlation multiplied by 100%. Bacterial community diversity, indicated by Shannon index, was calculated for rhizosphere and bulk soil.

Substrate-induced respiration (SIR) was measured by Izermeier technique to determine metabolically active microbial biomass in soil (I, II). The microbial biomass C was calculated according to Beck et al. (1996; II). Soil microbial respiration rate (basal respiration – BAS) was measured by titration according to Öhlinger (1996; II, III). The metabolic quotient (BAS/SIR), indicating carbon availability in the soil (Dilly et al. 2000; Weixin et al. 1996), was also calculated (II, III). Microbiological methods are thoroughly described in paper I.

## 2.7. Statistical data processing

Normality of variables was checked by Lilliefors and Shapiro-Wilk's tests. When the data deviated from normal distribution log- and root-transformations were applied where appropriate. Concerning other ANOVA assumptions – the homogeneity of the group variances was controlled by Levene's test; also plot of group means versus standard deviations was produced and checked. Differences between stand means were checked by Tukey unequal N HSD test (I–III; V); if assumptions for ANOVA (the same for multiple comparison of means) were not fulfilled, nonparametric Kruskal-Wallis multiple comparison of mean ranks was applied (I). To compare means of two independent groups, t-test was used (IV). To compare dependent samples, pairwise t-test was used (I). Sensitivity of a EcM root morphological parameter to site conditions (I) or

stand age (V) was evaluated as variation coefficient of stand means. Multiple stepwise regression analysis was used to find and estimate the effects of age and/or site properties (leaf and soil chemical attributes) on studied EcM morphological parameters (I, V). Gamma correlation was used to find relations between short root morphological parameters and exploration types of EcM morphotypes (V). Partial correlation analysis was used to remove the influence of age for finding relations between EcM root morphology and site properties in the natural forest chronosequence (V). Differences between trendlines of morphological parameters were detected by comparing the slopes of trendlines by t-test after linearizing the curvilinear trendlines. The significance level of  $\alpha=0.05$  was set in all cases. To ordinate morphological parameters and stands, principal component analysis (PCA; I, II) or redundancy analysis (RDA; V) was used. Data management and analysis were performed using STATISTICA 7.1 (StatSoft Inc 2002) and CANOCO ver. 4.52 (ter Braak and Šmilauer 2002) software.

## 3. RESULTS AND DISCUSSION

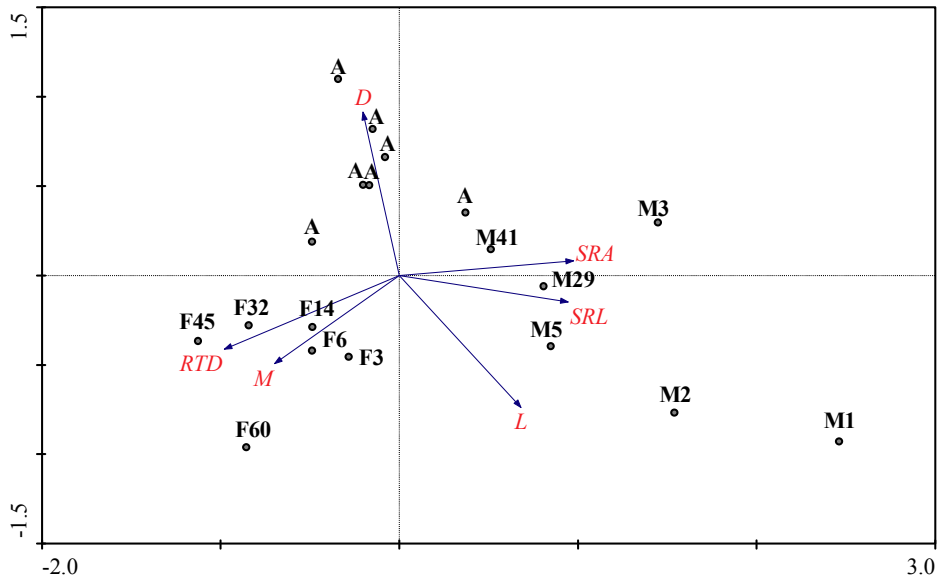
### 3.1. EcM root morphology of silver birch

EcM root morphology of silver birch was studied in stands of three forest categories: growing on natural forest land of *Oxalis* site type (natural forest), previous agricultural land (agricultural land), and in reclaimed opencast oil shale mine area (mine area).

In natural forest and mine area, investigations were carried out in the stand chronosequences to use the data for modelling the dynamics of root and leaf traits (morphology, nutrient concentration) during stand development. Previous land use affected significantly EcM morphology of silver birch – the studied stands formed separate groups according to forest category in a correlation biplot based on redundancy analysis of EcM root morphological parameters (Fig. 1). The site groups were ordered mainly by the first axis. For both chronosequences, the age effect appeared similar, and stands were ordered by stand age along the first axis as a rule (Fig. 1, II, and V). The first axis was best related to EcM root SRA, SRL, and root tissue density. That pattern for SRL and SRA is common for ordinations of EcM root morphological parameters (I, II, V, Ostonen et al. 2007b). The second axis was best related to EcM root diameter followed by length.

The stands of the mine area chronosequence had the biggest between-site variation because great changes occurred in soil conditions during stand development in primary succession (Table 2, Fig. 1 in II). One forest stand on agricultural land (Reigi) which lay on the ordination plot (Fig. 1) nearest to the mine area stands had similar soil type as older mine area stands.

Most probably, as it can be seen from the ordination plot (Fig. 1), the stands of mine area and agricultural land are likely to develop towards the natural forest stands.



**Figure 1.** Correlation biplot based on redundancy analysis of EcM root morphological parameters of silver birch stands growing in different forest sites. The relative eigenvalues of axis 1 (horizontally) and axis 2 (vertically) are 39 and 8%, respectively. The class variables are indicated by circles. Abbreviations for class variables: natural *Oxalis* site type forest – F, previous agricultural land – A, reclaimed oil shale mine area – M. Numbers after letters indicate stand age. Forests on abandoned agricultural land belonged to the same age class (mean age 12 years). Abbreviations for dependent variables: SRL – specific root length, SRA – specific root area, L – length, M – mass, RTD – root tissue density, D – diameter.

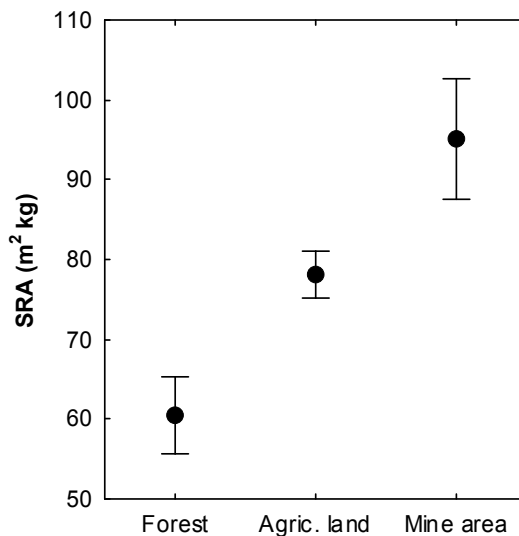
### 3.1.1. Site effect on EcM root morphology of young silver birches

To estimate the forest-category-related differences and diminish stand or tree age effect on EcM root morphology, only data for young (5–14-yr-old) silver birch stands, belonging to different forest categories, were compared (Table 2, Fig. 2). The 6- and 14-yr-old natural forest stands formed one group (Table 2) because means of morphological parameters did not differ significantly (Tukey unequal N HSD) between those age classes.

**Table 2.** EcM root morphological parameters (mean  $\pm$  SE) in young silver birch stands grouped according to previous land use. (Sample size n=18–24 per stand). Different letters indicate significant differences between site types by unequal N HSD test ( $p < 0.01$ ).

Forest category	Stand age (yrs)	SRL ( $\text{m}^2\text{kg}^{-1}$ )	Tissue density ( $\text{kg m}^{-3}$ )	Diameter (mm)	Length (mm)
Natural forest	6, 14	$68.8 \pm 3.2^a$	$235 \pm 5^b$	$0.290 \pm 0.006^a$	$1.05 \pm 0.04^a$
Previous agricultural land	8–14	$74.7 \pm 2.0^a$	$157 \pm 3^a$	$0.340 \pm 0.005^b$	$0.98 \pm 0.02^a$
Reclaimed mine area	5	$112.6 \pm 9.4^b$	$154 \pm 3^a$	$0.281 \pm 0.009^a$	$1.49 \pm 0.05^b$

Among the studied EcM root morphological parameters, SRA was the most sensitive parameter to site conditions in young silver birch stands – mean SRA differed between all studied sites (Fig. 2) and was ordered:  $\text{SRA}_{\text{natural forest}} < \text{SRA}_{\text{agric. land}} < \text{SRA}_{\text{mine area}}$ .



**Figure 2.** EcM root SRA for young (5–14-yr-old) silver birch stands for different site categories according to previous land use: natural forest, agricultural land, and mine area. Vertical bars denote 0.95 confidence intervals.

Also the EcM root SRL was the highest in birch stands in mine area as a result of both the low tissue density and thin roots. The highest tissue density of EcM roots was found for birches in natural forest; EcM root diameter was greatest on agricultural land compared to the other forest categories. Mean ( $\pm$  SE) EcM root

tip frequency per mass unit ( $\text{mg}^{-1}$ ) was highest for young birches in natural forest ( $68.9 \pm 3.7$ ) and did not differ between mine area ( $76.6 \pm 6.4$ ) and agricultural land ( $79.4 \pm 2.5$ ). However, the mean root tip frequency per mass unit was the most sensitive EcM root morphological parameter inside a forest category (I, II, V).

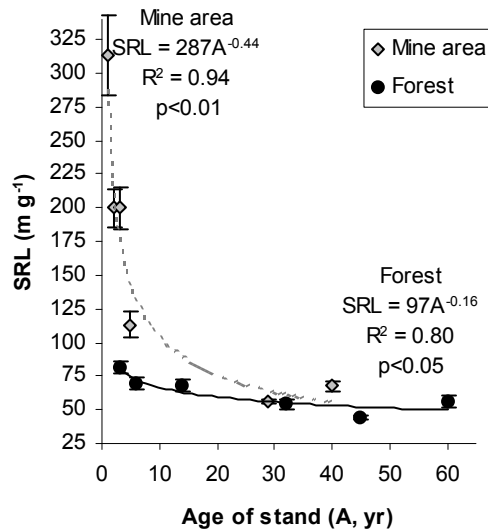
The effect of site properties on EcM root morphology can be caused by a variation of several factors including soil physical characteristics and nutrient availability, different communities of root-colonising EcM (discussed in Chapter 3.6.), and regeneration type (planted or natural) that strongly affects stand development. Soil mechanical impedance can initiate a strong change in the morphology of the roots. Generally, root diameter behind the root tip increases and root elongation decreases with increasing soil strength (Veen and Boone 1981; Hodge et al. 2009; Makita et al. 2011). The studied site types differed considerably by soil physical properties. Due to ploughing, the topsoil of the agricultural lands has higher bulk density than soils in *Oxalis* site type natural forest. EcM root diameter was indeed greater on agricultural land than in natural forest. Mine areas, on the other hand, are extremely stony, but the developing soil layer, where roots grow, has a relatively high percentage of organic matter. Organic matter % (estimated as loss on ignition) of fine earth in young birch stands decreased as follows: natural forest (6.3) > mine area (4.2) > agricultural land (3.0).

The improved nutrient supply decreases fine root SRL of trees including silver birch (review by Ostonen et al. 2007a; Kalliokoski 2010). This idea is supported also by our data in the concluding Table 2, which shows that EcM root SRL of young birches was the highest in mine area, where trees grow in harsh conditions (high pH limiting nutrient, in particular phosphorus, uptake and high stoniness, which affects water-holding capacity and soil NPK pools negatively). Under growth-limiting conditions, construction costs per root length (SRL) should be maximized to enable a large soil volume to be exploited with a small root construction cost (Ryser 2006). But also inside the site type, EcM root SRL and SRA of silver birch were negatively related to leaf N% on agricultural land (III) and in natural forest (IV). Hence, high SRL and SRA may compensate for low nutrient supply rates and could be the root nutrition stress indicators for silver birch.

### **3.1.2. EcM root morphological parameters in chronosequences of silver birch stands**

Stand age had a significant effect on EcM root morphology in natural forest as well as mine area – clear age-related dynamics appeared for most measured morphological parameters. EcM root mean diameter, mass, and EcM root tissue density increased with stand age, whereas SRA, SRL,  $\text{RTF}_M$  decreased with stand age (Fig. 3.; Table 3 and Fig. 4b in II, Fig. 1 in V) for both chrono-

sequences. However, EcM root length decreased with stand age in mine area only (Fig. 4a in II).



**Figure 3.** EcM root specific root length (SRL) in chronosequences of silver birch stands growing in *Oxalis* site type natural forest (Forest) and reclaimed oil shale mining area (Mine area). Bars indicate standard errors of mean.

Concerning time scale of plant response to environment, the definitions of stress, acclimation and adaptation given in Lambers et al. (2006) were used. The phenomenon that EcM root parameters had similar age trends for both chronosequences is most probably an expression of adaptations – the evolutionary response that results from genetic changes in populations leading to morphological and physiological compensation for the decline in performance caused by stress. Higher EcM root SRL and SRA of younger trees can ensure their superior competitive ability in higher stress conditions. Generally, for naturally regenerated forests, competition between trees is higher in younger stands, where stand density is higher leading to stronger root and light competition; hence, young trees have to apply efficient compensative strategies.

Linearised trendlines for natural forest and mine area differed for EcM root SRL (Fig. 3), SRA and diameter (intercepts and slopes of regression lines were compared by t-test, results not shown,  $p < 0.05$ ). These significant differences between time courses for the forest categories of an EcM root morphological parameter represent acclimation and are related to responses to different environments (see Chapter 3.1.1).

EcM root tissue density increased with stand age in natural forests linearly, but in mining area logarithmically; however mean root tissue density values for all age classes in natural forest were higher than those in mine area (Table 3 in



II, Fig. 1f in V). The age-related increase of root tissue density and diameter may be caused by a lower root turnover rate of EcM roots of older trees because root tissue density and diameter are higher for roots with a longer life-span (Eissenstat and Yanai 1997; Withington et al. 2006). In young stands, EcM root tissue density and also diameter are lower due to fast growth and expansion of root system at young age.

The age-related changes in EcM root morphology in a chronosequence were much greater for oil shale mine area than for natural forest because soil conditions of mine refuse had changed considerably during primary succession (Table 1).

### **3.2. Soil and leaf chemical properties in chronosequences**

The impact of stand age on soil chemical characteristics was different in the chronosequences of natural forest and mine area. Soil N% increased an order during 30 years of soil succession in mine chronosequence; in natural forest chronosequence, on the contrary, soil N% decreased with increasing age (Table 1). In natural forest, soil N was especially high in the youngest stand partly as a result of increased decomposition and N mineralisation after clear-cut disturbance (White et al. 2004). Besides, Umbric Gleysol – the soil type of the youngest stand – may also have naturally higher N% than other soil types of the studied natural forests. Along the rest of forest chronosequence, soil N% diminished modestly.

Soil pH of both chronosequences had also contrary dynamics: soil pH decreased with time in mine chronosequence with initially alkaline soil and increased in natural forest chronosequence with acidic soils. It was revealed that the optimal soil pH for silver birch should remain between 4 and 5 – rhizosphere processes do not change the rhizosphere pH in this pH range (Fig. 2 in paper I). Hence, rhizosphere processes in silver birch stands contribute to soil pH decrease in mine area with alkaline soil, and increase soil pH in natural forest with acidic soil to improve tree mineral nutrition. Therefore, changes in soil chemical properties in a birch chronosequence depend on initial soil conditions of the site.

The chemical composition of plant tissues is affected by many abiotic and biotic factors; it reflects both the soil composition at a particular site and the ability of the plant to accumulate or exclude excessive uptake of certain elements (Ericsson 1994). The effect of age on tree mineral nutrition was clearly revealed in the natural forest chronosequence: leaf N and K decreased, and P increased with stand age (Fig. 4 in paper V). Similarly, leaf P increased with stand age in young silver birch chronosequence (2-, 4-, 7-yr-old stands) in mine area despite of decrease in available (lactate-soluble) soil P (Kuznetsova et al. 2010). One reason for increasing leaf P with age may be the response to

improved soil pH for P uptake. Soil pH in forests remained under 4, but increased with age; in mine area, soil pH decreased during the first 7 years by 0.4 units and during the first 30 years by one pH unit. It is generally accepted that inorganic phosphorus availability to plants is maximized when  $\text{pH}_{\text{KCl}}$  remains between 4.5 and 6.5.

Our finding that leaf N% declined with tree age (and size) agrees with results of a birch study by Thomas (2010), where a similar trend was found for leaf N% of *Betula alleghaniensis* Britt. High leaf N indicates high leaf mass-based net photosynthesis (through an increase in the pool of enzymes and other proteins involved in light interception, light conversion, and carbon fixation), nitrogen uptake rate and growth and maintenance respiration (Reich et al. 1998; Thornley and Cannell 2000; Wright and Westoby 2001; Delagrange 2011). Therefore higher leaf N at young age allows birches to grow rapidly in order to survive the strong competition for light and other resources. The retardation of height growth rate with increase of tree size and the levelling off or decrease of net primary production (NPP) in old forests seems rather to be a consequence of the complex interaction between environmental constraints, physiological compensation, evolutionary adaptation, population- and community-level processes and ecosystem development (Kutsch et al. 2009). Age-related decrease of main nutrient – leaf N concentration could be caused by many reasons including (1) decreased nutrient uptake in older stands because more nutrients are sequestered in woody biomass and less nutrients are available in the soil (Gower 1996), (2) older trees use nutrients more efficiently than smaller ones (Siddiqui et al. 2009), and (3) demand of older trees for leaf N is smaller. Leaf K% in *Oxalis* site type silver birch forests was below growth-limiting concentrations across the chronosequence according to Ingestad and McDonald (1989). The K deficiency may also be caused by leaching of K from acidic soils of stands in the natural forest chronosequence.

### **3.3. SLA in the *Oxalis* site type natural forest chronosequence**

The specific leaf area decreased in natural forest chronosequence (Fig. 3 in V); this ontogenetic shift is in accordance with previous studies for deciduous tree species. As light conditions at the time of foliage formation have generally been regarded as the primary factor influencing SLA in both hardwoods and conifers (Weiskittel 2008), decline in SLA along the studied naturally regenerated birch chronosequence may also be related to decreasing stand density – competition for light between trees is higher in younger denser stands and light interception is maximized with high SLA there. Our assumption that dynamics of leaf and root specific areas in the birch chronosequence may be similar was clearly proven – both parameters decreased with tree age. Decreased specific leaf area is a main factor associated with decline in the relative growth rate (RGR) (e.g.

Lambers and Poorter, 1992; Reich 1998; Imada et al. 2010); consequently, EcM root SRA should decrease as well when tree growth slows down.

### **3.4. EcM root N**

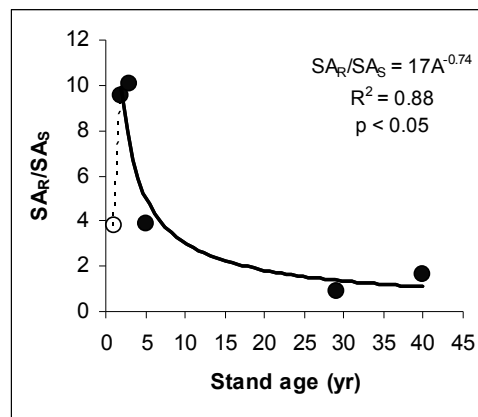
Root N concentration of deciduous trees is positively related to root respiration (Pregitzer et al. 1998; Reich et al. 1998; Makita et al. 2009) as well as to nutrient acquisition rate (Reich et al. 1998), thus to the general physiological activity. As roots with high N concentrations have higher respiration rate, fine root construction and maintenance costs are inversely related (Pregitzer et al., 1998, 2002), which affects fine root longevity. Root N is a key factor in understanding C costs of maintaining fine roots (Pregitzer et al., 2002). EcM root N% was a stable and relatively autonomous root parameter for the studied silver birch stands – site properties affected root N concentrations insignificantly. EcM root N concentration varied between 2.14 and 3.16% in natural forest chronosequence, from 2.05 to 2.40% in mine area chronosequence, and from 2.24 to 2.46% in young stands on abandoned agricultural land. No age trend of root N% appeared in the studied chronosequences. EcM root N did not correlate with root morphological parameters nor with leaf N.

EcM root N% of 3-year-old silver birches was similar for natural forest (2.17) and for mine area (2.23); the same range was revealed for EcM root N% of 3-year-old *Quercus robur* L. ( $1.97 \pm 0.29$ ) in Trocha et al. 2010. Generally, root and leaf N concentrations both reflect the ability to capture resources through enzymatic processes (Liu et al. 2010). Stable EcM root nitrogen status was little affected by soil properties or tree age, most probably because EcM roots are first served by assimilated nutrients and achieve first the necessary N concentration. To ensure photosynthesis, leaf N% in young silver birch stands on abandoned agricultural land was almost always higher than root N%. In natural forest chronosequence, leaf N% was higher than root N% only for two youngest age classes (3 and 6 yrs). There are indications that N% in silver birch leaves or EcM roots depends on whether the leaf- or root-related physiological processes need more N.

### **3.5. Rhizosphere effect in relation to soil properties and stand age**

Rhizosphere effect on summed bacterial functional activity (hereafter bacterial activity) was measured in mine area chronosequence (II) and young birch stands on previous agricultural land (I). For strongly acid forest soils in silver birch stands of *Oxalis* site type, the importance of rhizosphere bacteria should be lower than for EcM fungi (Bååth and Anderson 2003), therefore only colonization of short roots by EcM fungi was studied.

In young silver birch stands of agricultural land, rhizosphere effect on soil bacterial activity (assessed as summed functional activity ratio  $SA_R/SA_S$ ) was strongly related to bulk soil pH – in case of lower soil pH, birch supports more rhizosphere bacteria and the rhizosphere effect is more pronounced (Fig. 4 in paper I). However, when bulk soil pH was  $\geq 5$ , the ratio  $SA_R/SA_S$  did not decrease any more, i.e. the rhizosphere effect stayed at a stable level. Phillips and Fahey (2006) found that rhizosphere effect on net N mineralization and phosphatase activity was much lower in soils with  $pH(H_2O) > 4.3$  for EcM tree species and suggested that soil pH and its relation to nutrient availability may also influence the magnitude of rhizosphere effects. In young birch forests on agricultural land, rhizosphere effect on bacterial activity correlated also positively with EcM root N concentration (Fig. 5 in I). Most probably there is a positive feedback while root N concentration indicates general physiological activity of root. Rhizosphere effect on bacterial activity of young birches on agricultural land varied three times (1.4–4.7) due to broad soil pH gradient of stands and was comparable to rhizosphere effect calculated (according to trendline function in Fig. 4) for young (8–14-yr-old) birches of mine area (2.4–3.6).



**Figure 4.** Dynamics of rhizosphere effect, indicated by  $SA_R/SA_S$ : summed bacterial activity in rhizosphere divided by that in bulk soil. The white dot indicates  $SA_R/SA_S$  in the planting year.  $SA_R/SA_S$  value in the first year was affected by the transplant shock, and therefore it was excluded from trendline data; A – stand age. (Rosenvald et al. 2010)

In the silver birch chronosequence of reclaimed oil shale mine area, rhizosphere effect on soil bacterial activity had the highest values in the second and third year after planting, when surviving plants have overcome planting shock, and decreased hyperbolically after that (Fig. 3). In the middle-aged stands, where soil conditions are greatly improved and pH has decreased by a unit, rhizosphere effect on soil bacterial activity was low because the need for rhizosphere

support is not as important there. During the first year fine roots exploit the oil shale mining spoil extensively, and there is a deficit in the assimilates allocated below ground.

Rhizosphere effect on pH was revealed both in mine chronosequence and on agricultural land. Birch rhizosphere pH was lower than bulk soil pH (up to 1.5 units) across the mine area chronosequence and in most young birch stands on agricultural land. On agricultural land, rhizosphere pH was higher than soil pH in stands with acidic soils (pH<5).

Diversity of bulk soil bacterial community increased simultaneously with soil formation under silver birch in mine area – from 3.2 (in 2-year-old stand) to 3.7 (in 40-year-old stand) and was comparable with that in young birch stands of arable land (3.4–3.6). Such an increase in microbial community diversity in mine area probably reflects the diversification of soil conditions during the soil formation process. There are two main factors that contribute to this process – change in soil chemical conditions (a greater number of different organic substrates) and increased habitat heterogeneity.

Lower diversity of bacterial communities in rhizosphere than in bulk soil is commonly found for several grass species (Berg and Smalla 2009), indicating that bacterial community structure was shaped by rhizodeposition, which forms a distinctive bacterial population in the rhizosphere compared to bulk soil. This was also the case in birch stands on agricultural land (except in Reigi), but not in birch stands in mine area, where the diversity of bacterial communities was always higher in rhizosphere than in bulk soil. However, that difference (i.e. rhizosphere effect on bacterial diversity) decreased linearly with increasing stand age, and according to that relationship, after a stand age of 53 years, diversity in rhizosphere should be continuously lower than in bulk soil.

The similarities between rhizosphere and bulk soil bacterial communities were lower in birch stands in mine area (33–42%) than on arable land (45–72%).

### **3.6. EcM community of silver birch in *Oxalis*-type natural forests**

The pilot study of dominating EcM fungal species or morphotypes of silver birch was carried out in the natural forest chronosequence for three stand ages: 6, 14, 32 yrs. (Table 3 in V). Molecular analyses revealed 33 EcM fungal species among the dominant morphotypes in three studied age classes. EcM community structure differed between the age classes of the chronosequence; there were only three EcM species and one morphotype (*Tomentella sublilacina*, *Tomentella badia*, *Cenococcum geophilum*, *morph2*) which we found in more than one stand. Twieg et al. (2007) found 82 EcM fungal species in total colonising paper birch in the chronosequence (5, 26, 65, and 100 yrs) of mixed forest of Douglas-fir (*Pseudotsuga menziesii* Mirb.) and paper birch (*Betula*

*papyrifera* Marsh.). Similarly to our results, Twieg et al. (2007) found that ECM fungal community compositions differed among all pairs of age classes; however, they were similar in 65- and 100-yr-old stands.

The effect of exploration type on EcM root morphology was revealed. The four exploration types were ordered: contact (1), short- (2), medium- (3), and long-distance (4). A weak negative correlation was found between exploration type and EcM root tissue density as well as mass; gamma correlation coefficients (Gamma R) were  $-0.32$  and  $-0.30$ , respectively ( $p < 0.01$  in both cases). Exploration type correlated positively but also weakly with SRA and  $RTF_M$  (Gamma R= $0.31$  and Gamma R= $0.30$ , respectively;  $p < 0.01$  in both cases).

Despite the fact that most of dominating EcM root colonising species differed for the studied stands, distribution of the different EcM exploration types affecting the nutrient acquisition efficiency of EcM roots (Agerer 2001) was similar regardless to stand age. However, the difference in nutrient uptake between similar exploration type forming EcM fungi cannot be excluded. Nevertheless, we set a hypothesis that the possible effect of EcM fungal community on mineral nutrition of silver birches across the chronosequence is probably similar as well, and the observed differences in root morphological characteristics are driven mainly by ontogenesis of the tree. Verification of this hypothesis needs further studies.

### **3.7. EcM root adaptations and rhizosphere effect of silver birch compared to other tree species**

The comparison of silver birch with the other studied pioneer tree species – black alder, Scots pine – was carried out in stands of reclaimed oil shale mining area (III, IV). After one year of growth in harsh conditions of mining refuse, roots of silver birch were thinner, lighter in mass, and had lower tissue density than roots of black alder and Scots pine (III). Also SRL, SRA, and  $RTF_M$  were remarkably higher than for the other studied species. High morphological plasticity of silver birch fine roots has also been shown by Kalliokoski et al. (2010), who compared birch with Norway spruce or Scots pine. The higher EcM root SRL and SRA for silver birch indicate that a large assimilating area per mass unit is formed leading to a smaller EcM root cost. Also the biomass proportion of fine roots and leaves for one-year-old stands was significantly smaller for silver birch than black alder (III). For reclaimed mining area, the survival of silver birch seedlings after one growing season was lower (64%) than that of seedlings of black alder (93%) and Scots pine (83%). Probably the lowest survival and also the smallest relative height increment of silver birch are due to its very thin and highly ramified roots having low water conductivity (Sellin and Kupper, 2005), which causes shortage of assimilates in the first year.

The overall dynamics of rhizosphere effect in 1-, 4-, 27-yr-old stands was similar for silver birch and black alder in reclaimed oil shale mining area. However, the rhizosphere effect on bacterial activity for 4-year-old alders ( $SA_R/SA_S=23$ ) was more than twice higher than that for 4-year-old birches ( $SA_R/SA_S=10$ ) (IV). Compared to black alder, silver birch was oriented more towards fine-root adaptations than support of rhizosphere communities.

## 4. CONCLUSIONS

The overall objective of the thesis was achieved. Most of the hypotheses were verified and proved. During the research there was a need to present new hypotheses; the respective conclusions are presented without hypotheses' numbers. The results of the thesis provide cognitive basis for formulating new hypotheses concerning fine root and rhizosphere processes under changing environmental conditions.

- EcM morphology and rhizosphere effect were influenced by site properties especially by previous land use as well as tree age (Hypothesis 1, 2).
- The ontogenetic changes in EcM root morphology occur irrespective of site properties – EcM root morphological parameters had a similar general pattern in natural forest and reclaimed oil shale mine area chronosequence despite the very different site conditions.
- Temporal changes in EcM root morphology occurred faster in younger age, before the age of 5–10 years (Hypothesis 4).
- The functional balance between physiology and morphology of leaves and roots, as functionally homogeneous and most active parts of trees, appeared during stand development in natural forest. The specific leaf area (SLA) and specific EcM root area (SRA) as well as leaf N decreased simultaneously with tree age probably, as a result of down-regulation of growth (Hypothesis 5).
- Age-related decrease of silver birch SRL and SRA was caused both by increasing EcM root diameter and tissue density. Hence, probably EcM roots of older trees have longer life span.
- High EcM root SRL and SRA can be considered as root N nutrition stress indicators for silver birch – both increased in case of low leaf N% (Hypothesis 1).
- The exploration type of EcM fungi significantly affected EcM root morphology. Despite the fact that most of the dominating EcM root colonising species differed between the studied silver birch stands, distribution of the different EcM exploration types affecting nutrient acquisition efficiency was similar regardless of stand age.
- The rhizosphere effect was higher by lower pH and higher root N% on agricultural land. Rhizosphere processes in silver birch stands contribute to soil pH decrease in mine area with alkaline soil, and soil pH increase in natural forest with acidic soil to improve tree mineral nutrition. In young birch forests on agricultural land, rhizosphere acidification occurred in the studied stands when bulk soil pH(KCl) > 5.
- Compared to black alder, silver birch was oriented more towards fine-root adaptations than support of rhizosphere communities (Hypothesis 6).
- Strategies improving root nutrition – morphological adaptation and rhizosphere effect – depended on different environmental factors, which means they are based on different regulatory mechanisms.



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## 6. SUMMARY IN ESTONIAN

### Imijuuremorfoloogiat ja risosfääriprotsesse mõjutavad tegurid arukaasikutes

Mineraaltoitainete, eelkõige lämmastiku kättesaadavus on peamine metsaökosüsteemi produktiivsust ja süsiniku salvestumist limiteeriv tegur. Peenjuurtesse ( $D < 2$  mm) võib minna kuni 75% metsade netoproduksioonist (Vogt 1991), mistõttu on peenjuurteil süsinikuringes väga oluline roll. Ektomükoriissete puude, sh arukase mineraaltoitumise seisukohalt on peenjuurestikus olulisimad imijuured, mis seenpartneri ehk ektomükoriisaseene niidistiku abil omastavad mullast vett ja mineraaltoitaineid. Ektomükoriisid imijuured on funktsionaalselt homogeenne ja füsioloogiliselt kõige aktiivsem juurestiku osa. Ektomükoriissete juurte funktsionaalset efektiivsust iseloomustab eelkõige nende eripind (juurepind massiühiku kohta) ja -pikkus (juurepikkus massiühiku kohta) ning nende füsioloogilist aktiivsust peegeldab lämmastikisisaldus (Lõhmus et al. 1989; Eissenstat and Yanai 1997; Reich et al. 1998; Ostonen et al. 2007a; Richardson et al. 2009; Liu et al. 2010).

Mineraaltoitainete omastamiseks mullast peavad juured optimeerima oma morfoloogilisi parameetreid ja mõjutama oma lähiümbrust – risosfääri (Hinsinger et al. 2009). Risosfäär on õhuke juurt ümbritsev mullakiht, mida mõjutab juurte elutegevusega kaasnev risodepositsioon ehk süsinikuvoog mulda. Risodepositsioon võib moodustada isegi kuni 44% fotosünteesi käigus seotud süsinikust (Bais et al. 2006), kuid määrava tähtsusega on siin juureeritised, mis on mikroobidele kergesti omastatavad süsinikuallikad. Tänu risodepositsioonile on juurtoitumist soodustavad mikroobsed protsessid, nagu mineralisatsioon ja ensüümide aktiivsus risosfääris suuremad kui mullas (Radersma and Grierson 2004; Liu et al. 2006; Phillips and Fahey 2006; Bader and Cheng 2007; Mueller and Shann 2007; Zhao et al. 2007) – sellist erinevust nimetatakse risosfääri-efektiks.

Vastavalt optimaalsuse teooriale on talitlevate juurte jaoks määrav nende kulude/tulude suhe. Piisava mineraalse toitumise tagamiseks peavad puud investeerima maa alla assimilaate vastavalt evolutsiooniliselt väljakujunenud adaptiivsetele strateegiatele:

- a. ekstensiivne – juurte biomassi, pikkuse ja pindala suurendamine, mis samaaegselt suurendab risosfääri;
- b. intensiivne – imijuurte morfoloogilised adaptatsioonid ja risosfääri mikroobikoosluste toetamine (Lõhmus et al. 2006b).

Kui ekstensiivse strateegia tähendab peenjuurestiku suurenemist, siis intensiivne strateegia baseerub imijuurte massiühiku efektiivsemal kasutamisel ehk morfoloogilistel adaptatsioonidel. Lisaks juuremorfoloogia muutmisele saab puu juurtoitumist parandada ka risosfääriefekti kaudu – näiteks risosfääri suunduva süsiniku kogust ja koostist muutes. Risosfääriefekti saab mõõta risosfääri- ja mullaparameetrite suhte või vahena.

Doktoritöö põhieesmärk oli (I) analüüsida arukase (*Betula pendula* Roth.) kui Põhja-Euroopas levinud ja majanduslikult olulisima lehtpuuliigi imijuurte morfoloogilisi adaptatsioone ja risosfääriefekti erinevates kasvukohatingimustes; (II) välja selgitada puude vanuse mõju imijuurte morfoloogiale ja risosfääriefekti avaldumisele seoses puu mineraalse toitumusega.

Kasvukoha mõju kaskede ektomükoriisete imijuurte morfoloogiale ja risosfääriefektile analüüsiti endistel põllumaadel kasvavates noortes kaasikutes. Vanuselisi muutusi ektomükoriisete imijuurte morfoloogias ja risosfääriefektis uuriti jänesekapsa kasvukohatüübis looduslikult uuenenud kaasikute ja tasan-datud põlevkivikarjääridele istutatud kaasikute vanuseridades. Bakterite aktiivsuse puhul käsitleti risosfääriefekti kui puu toetust risosfääribakteritele. Toitainete kättesaadavust puistus hinnati lehe lämmastiku-, fosfori- ja kaaliumi-kontsentratsioonide ja juure füsioloogilist aktiivsust juure lämmastikukontsentratsioonide kaudu. Mükoriisat moodustava seeneliigi mütseelitüübi („exploration type“) mõju imijuurte morfoloogiale hinnati metsamaa kaasikute vanusera puistutes.

Kasvukoht ja puu vanus avaldasid olulist mõju nii imijuurte morfoloogiale kui ka risosfääriefekti avaldumisele. Imijuurte eripind ja eripikkus olid suuremad väiksema lehelämmastiku, seega halvema lämmastikutoitumuse korral nii endistel põllumaadel kui ka metsamaal kasvavates kaasikutes. Kase imijuurte morfoloogia dünaamika oli sarnane nii happelisel metsamaal kui ka lubjakivirikkal karjääripuistangul kasvavate puistute vanuseridades, kusjuures muutused imijuurte morfoloogias toimusid kiiremini nooremas eas. Mõlemas kaasikute vanuseraas kahanesis imijuurte eripind ja eripikkus puu vanusega, kuna imijuurte kudede tihedus ja diameeter suurenesid puu vanuse kasvades. Metsamaa arukaasikute vanuseraas mõõdeti lehtede eripinnad ja lämmastikukontsentratsioonid, mis samuti kahanesisid puu vanuse kasvades. Tõenäoliselt olid vanemate kaskede imijuurte suurem kudede tihedus ja diameeter tingitud nende pikemast keskmisest elueast (Eissenstat and Yanai 1997; Withington et al. 2006). Seega olid kiiret kasvu, kõrget efektiivsust ja füsioloogilist aktiivsust näitavad lehe- ja juureparameetrid suuremad nooremates puistutes ning võib järeldada, et lehtede ja imijuurte morfoloogia dünaamika puistu arengu vältel on kooskõlas.

Mükoriisa seenpartneri mütseelitüüp mõjutas imijuurte morfoloogiat looduslikes jänesekapsakaasikutes oluliselt. Samas oli erinevate mütseelitüüpide osatähtsus puistuti sama sõltumata puistu vanusest. Kuidas mütseelitüüp võiks avaldada mõju puude mineraaltoitumisele erineva vanusega puistutes, vajab täiendavaid uuringuid.

Mulla pH suurenes happelistel metsamuldadel kasvavate arukaasikute vanuseraas ning kahanesis aluselise pinnasega põlevkivikarjääripuistute vanuseraas. Risosfääriefekt endistel põllumaadel kasvavates noortes arukaasikutes sõltus mulla pH-st – risosfääribaktereid toetati seda enam, mida happelisem (bakteritele ebasoodsam) oli kasvukoha muld ja mida suurem oli imijuurte endi lämmastiksisaldus. Kui morfoloogilist kohanemist mõjutas lämmastiku kättesaa-



davus (imijuurte eripind ja -pikkus olid suuremad väiksema lehelämmastiku korral), siis risosfääriefekt sõltus eelkõige mulla pH-st. Seega peaks nende adaptatsioonide regulatsioon põhinema erinevatel mehhanismidel. Võrreldes teiste selles aspektis uuritud lehtpuuliikidega kasutavad kased juurtootumise parandamiseks rohkem imijuuremorfoloogia varieerimist kui risosfääribakterite toetamist.

Antud doktoritöös hinnati esmakordselt arukase ektomükoriisete imijuurte morfoloogiliste parameetrite varieeruvuse piire ning analüüsiti ektomükoriisete imijuurte morfoloogiliste adaptatsioonide funktsionaalset rolli erinevates kasvu-kohtades ja seoses puistute vanusega. Käesoleva töö tulemused aitavad püstitada uusi hüpoteese juurte ja risosfääri adaptatsioonimehhanismide edasiseks analüüsiks ja on kasutatavad erinevate keskkonnamõtjude sealhulgas muutuva maakasutuse tagajärgede prognoosimisel arukaasikutes.

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## **PUBLICATIONS**

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1999–2005 University of Tartu, BSc in environmental technology  
1995–1999 Virumaa College, applied higher education, teacher of mathematics, physics and informatics  
1984–1995 I Secondary School of Kohtla-Järve

**Languages** Estonian (mother tongue), English, German, Russian

### Professional employment:

2006–... Institute of Ecology and Earth Sciences, Tartu University, Chair of Physical Geography and Landscape Ecology; specialist in environmental technology (0.5)

### Current grants and projects:

2008–2011 ESF grant No. 7452: “Fine root adaptation strategies in European coniferous and deciduous forests along a latitudinal gradient”  
2009–2012 ESF grant No. 7792: “Rhizosphere processes in carbon and nutrient cycling of free air humidity manipulated deciduous forest ecosystems”  
2008–2013 TF project 0180127s08: “Material cycling of landscapes in changing climate and land use conditions and ecotechnological control thereof”.

### Field of research

Rhizosphere processes in silver birch stands

## Publications

### Journal articles indexed by ISI Web of Science

- Rosenvald K, Ostonen I, Truu M, Truu J, Uri V, Vares A, Lõhmus K (2011) Fine-root rhizosphere and morphological adaptations to site conditions in interaction with tree mineral nutrition in young silver birch (*Betula pendula* Roth.) stands. *European Journal of Forest Research*, DOI: 10.1007/s10342-011-0492-6,
- Rosenvald K, Kuznetsova T, Ostonen I, Truu M, Truu J, Uri V, Lõhmus K (2010) Rhizosphere effect and fine-root morphological adaptations in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas. *Ecological Engineering*, DOI: 10.1016/j.ecoleng.2010.05.011
- Kuznetsova T, Rosenvald K, Ostonen I, Helmisaari H-S, Mandre M, Lõhmus K (2010) Survival of black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) seedlings in a reclaimed oil shale mining area. *Ecological Engineering*, 36(4), 495–502

### Articles/chapters in books (including collections indexed by the ISI Web of Proceedings)

- Lõhmus K, Kull A, Truu J, Truu M, Kaar E, Ostonen I, Meel S, Kuznetsova T, Rosenvald K, Uri V, Kurvits V, Mander Ü (2007) The reclamation of the North Estonian oil shale mining area. Mander Ü, Wiggering H, Helming K (ed.). Multifunctional land use: Meeting future demands for landscape goods and services (387–401). Berlin Heidelberg: Springer
- Lõhmus K, Truu J, Truu M, Kaar E, Ostonen I, Alama S, Kuznetsova T, Rosenvald K, Vares A, Uri V, Mander Ü. (2006) Black alder as a perspective deciduous species for reclaiming of oil shale mining areas. Brebbia, C. A.; Mander, Ü. (ed.). Brownfields III. Prevention, Assessment, Rehabilitation and Development of Brownfield Sites (87–97). Southampton, Boston: Wessex Institute of Technology Press (ISI)

### Other publications

- Lõhmus K, Truu J, Truu M, Kaar E, Ostonen I, Meel S, Kuznetsova T, Rosenvald K, Vares A, Kurvits V, Kanal A, Uri V (2010) Risosfääriprotsesside mõju metsaökosüsteemi kujunemisele erineva liigilise koosseisuga puistutes põlevkivikarjääride puistangutel. Kaar E, Kiviste K (Eds.). Maavarade kaevandamine ja puistangute rekultiveerimine Eestis (185–194). Tartu: Eesti Maaülikool

## Presentations

1. Rosenvald K, Truu M, Kuznetsova T, Ostonen I, Truu J, Uri V, Lõhmus K (2009): Rhizosphere, fine-root, and soil parameters in a silver birch stand chronosequence on reclaimed oil shale mining area. *6th International Symposium on Ecosystem Behaviour BIOGEOMON 2009, June 29–July 3, Helsinki, Finland.*
2. Rosenvald K, Truu M, Kuznetsova T, Ostonen I, Truu J, Uri V, Lõhmus K (2009): Dynamics of fine-root, rhizosphere and soil parameters in a silver birch stand chronosequence on reclaimed oil shale mining area. *7th ISRR Symposium 'Root Research and Applications', September 2–4, Vienna, Austria*
3. Rosenvald K, Truu M, Truu J, Vares A, Uri V, Ostonen I, Lõhmus K (2007): Factors affecting rhizosphere processes in young silver birch stands on abandoned agricultural land. *International Conference Rhizosphere 2, 26–31 August, Montpellier, France.*
4. Rosenvald K, Truu M, Truu J, Uri V, Vares A, Ostonen I, Lõhmus K (2008): Fine root adaptations in relation to tree mineral nutrition in young silver birch (*Betula pendula* Roth.) stands on abandoned agricultural lands. *Woody Roots and Ecosystem Services, 16–20 May, Lisbon, Portugal.*
5. Rosenvald K, Kuznetsova T, Lõhmus K, Ostonen I (2006). Root adaptations of one-year-old Scots pine, silver birch, black alder and hybrid larch seedlings in calcareous quarry detritus of oil shale mining refuse. *COST E38 Workshop: Roots, mycorrhizas and their external mycelia in carbon dynamics in forest soils, 9–13 September, Rovaniemi, Finland.*

# CURRICULUM VITAE

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### Haridus

2006–2011 Tartu Ülikool, doktoriõpingud  
1999–2005 Tartu Ülikool, BSc keskkonnatehnoloogia eralal  
1995–1999 Virumaa Kõrgkool, rakenduskõrgharidus matemaatika-, füüsika- ja informaatikaõpetaja erialal  
1984–1995 Kohtla-Järve 1. Keskkool

**Keelteoskus** eesti (emakeel), inglise, saksa, vene

### Teenistuskäik

2006–... Tartu Ülikooli Ökoloogia- ja Maateaduste Instituut, Loodusgeograafia ja maastikuökoloogia õppetool; keskkonnatehnoloogia spetsialist (0.5)

### Projektid

2008–2011 ETF grant 7452: “Peente juurte adapteerumisstrateegiad Euroopa okas- ja lehtpuumetsades põhja-lõuna gradiendil.”  
2009–2012 ETF grant 7792: “Risofääriprotsessid muudetud õhuniiskusega lehtmetsaökosüsteemide aineringes”  
2008–2013 Sihtfinantseeritav teema SF 0180127s08: “Maastike aineringe muutuvates kliima- ja maakasutuse tingimustes ning selle ökotehnoloogiline reguleerimine”

### Teadustöö põhisuunad

Risofääriprotsessid arukaasikutes

## Publikatsioonid

### Ajakirja-artiklid, mis on kajastatud Thomson Reuters Web of Science andmebaasis

- Rosenvald K, Ostonen I, Truu M, Truu J, Uri V, Vares A, Lõhmus K (2011) Fine-root rhizosphere and morphological adaptations to site conditions in interaction with tree mineral nutrition in young silver birch (*Betula pendula* Roth.) stands. *European Journal of Forest Research*, DOI: 10.1007/s10342-011-0492-6,
- Rosenvald K, Kuznetsova T, Ostonen I, Truu M, Truu J, Uri V, Lõhmus K (2010) Rhizosphere effect and fine-root morphological adaptations in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas. *Ecological Engineering*, DOI: 10.1016/j.ecoleng.2010.05.011
- Kuznetsova T, Rosenvald K, Ostonen I, Helmisaari H-S, Mandre M, Lõhmus K (2010) Survival of black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) seedlings in a reclaimed oil shale mining area. *Ecological Engineering*, 36(4), 495–502

### Kogumikuartikkel/ peatükk raamatus

- Lõhmus K, Kull A, Truu J, Truu M, Kaar E, Ostonen I, Meel S, Kuznetsova T, Rosenvald K, Uri V, Kurvits V, Mander Ü (2007) The reclamation of the North Estonian oil shale mining area. Mander Ü, Wiggering H, Helming K (Toim.). Multifunctional land use: Meeting future demands for landscape goods and services (387–401). Berlin Heidelberg: Springer
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### Muud publikatsioonid

- Lõhmus K, Truu J, Truu M, Kaar E, Ostonen I, Meel S, Kuznetsova T, Rosenvald K, Vares A, Kurvits V, Kanal A, Uri V (2010) Risosfääriprotsesside mõju metsaökosüsteemi kujunemisele erineva liigilise koosseisuga puistutes põlevkivikarjääride puistangutel. Kaar E, Kiviste K (Toim.). Maavarade kaevandamine ja puistangute rekultiveerimine Eestis (185–194). Tartu: Eesti Maaülikool



# DISSERTATIONES GEOGRAPHICAE UNIVERSITATIS TARTUENSIS

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