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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

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The present research was carried out in Scots pine, black alder and silver birch stands of different age in Estonia in 2004 to analyse fine root morphological adaptations in recultivated areas of opencast oil shale mining and semicoke hills. Morphological adaptation of short roots to corresponding soil conditions was specific for tree species. Functional root characteristics – SRA ($m^2\ kg^{-1}$), SRL ($m\ kg^{-1}$) and RTD ($kg\ m^{-3}$) differed significantly among coniferous and deciduous tree species, with lowest SRA and SRL values and highest RTD values being found in Scots pine. The means of short root SRA varied from 43 to 68 $m^2\ kg^{-1}$ for pine; from 79 to 155 $m^2\ kg^{-1}$ for alder and from 157 to 194 $m^2\ kg^{-1}$ for birch. The impact of tree age on short-root parameters was significant for deciduous tree species. Root parameters on semicoke areas depend on the microbial community, as proved by the bioaugmentation experiment.

Introduction

Every year opencast oil shale mining in Estonia creates substantial areas of alkaline (pH~8) wasteland that require recultivation. Until 2005, around 12 900 ha was mined, and 10 188 ha of that had been afforested. As a result of oil shale opencast mining, the relief is rugged, the soil heterogeneous and

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extremely stony, nitrogen and organic content of oil-shale mining spoil low [1]. Fast growing tree species yielding high-quality timber that is in higher commercial demand are preferred on mining spoil areas; these are usually silver birch and black alder [2]. Black alder has proved one of the most productive tree species in oil-shale mining areas [3, 4]. Up to the latest years mainly coniferous trees have been planted on oil-shale mining areas, the share of pine, birch, spruce, larch and black alder stands in restored forests forming 86%, 9%, 3%, 1.6% and 0.2%, respectively. Fire and insects endanger spread monocultures of *Pinus sylvestris*. However, among deciduous trees, alders have a number of advantages over conifers: increased N and P availability in soil under alders, faster growth and higher resistance to pests, diseases and fires [4]. In terms of adaptive strategy, in forest succession, silver birch is likely to be a typical primary colonist and ruderal species and also grows well in calcareous areas. Although Scots pine is also a pioneer species, it is mostly a stress-tolerator [5].

In the process of shale oil production in Northeast Estonia, semicoke is one manufacturing residue, 600 000 tonnes of which is dumped every year to form giant semicoke hills. These hills currently comprise 70–80 million tonnes of semicoke and cover 180-200 hectares of land. The hills rise up to 120 metres above the surrounding landscape and are a serious pollution source for the surrounding areas, since the leachate from the hills contains environmentally hazardous substances, including phenols. Plants began to be introduced on to these mounds in the 1970s. At Kohtla-Järve the planted area has reached 60 hectares. In recent years, mainly silver birch has been planted, as it grows well on semicoke hills, but also black alder and Scots pine, and other species. When dumped, pH of semicoke is ~12.6; over time that of the topmost layers falls, and green plants are able to begin growing on the substrate. Remediation of semicoke by phytoremediation and bio-augmentation has been studied on the basis of four grass species at semicoke depository in 2002, and the biomass of phenol-degrading bacteria and diversity of microbial community in semicoke has been estimated [6]. Since 2004 bioaugmentation was applied in young silver birch plantations.

Root systems of trees vary widely both within and between species, in different soil conditions and forest site types. The term “fine roots” has been used for roots <2 mm in diameter. The short roots are morphologically distinct from the rest of the root system [7]. The majority of alder, birch, and pine short roots, as for primary structure, are ectomycorrhizas (ECM). Since short-root functions, such as water and ion uptake, are closely related to root structure, different morphological and physiological parameters of short absorbing roots are potential indicators of the mineral nutrition of trees on forest soils. Short roots adapt to changes in soil conditions by differentiating their anatomical and morphological features, which develop in conjunction with genetic variability and environmental conditions [8, 9]. On the level of individual root and the entire root system, the most essential functional characteristics are specific root area (SRA; $m^2\,kg^{-1}$), specific root length

(SRL; m kg^{-1}) and root tissue density (RTD; kg m^{-3}) [9–13]. For characterising short-root morphology, mean diameter of short roots (D, mm) and mean length (L, mm), weight (W, mg) and volume of the root tip (V, mm^3) are also important. These parameters, especially SRL, and to a lesser extent SRA, have been used as indices of root benefit to root cost, assuming that resource aquisition is proportional to the length or surface area, and root cost (construction and maintenance) is proportional to mass [11, 14–16]. SRA has been reported to be larger and RTD lower in highly productive Norway spruce stands with favourable soil conditions [12]. The larger the SRA and/or SRL and the smaller the RTD, the more effective is the strategy of allocation of assimilates to the build-up of short roots [11, 12, 17]. However, the variation of short-root parameters can imply an impact of root life span. High SRL is found in young root systems [18], and RTD of short roots is smaller for young unsenescent roots. Root tissue density of ECM is influenced by fungal symbionts [12], because the proportion of the mantle to the root volume and biomass varies with different ectomycorrhizal morphotypes [19].

According to our previous results, short-root morphological parameters of black and grey alders, especially SRA, were in good accordance with measurements of size and activity of microbial communities according to respiration, assessment of microbial activity by Biolog Ecoplates and foliar assimilation efficiency of the above-ground part of trees [20]. Hence, interaction of roots and soil microbial communities should be especially important in harsh site conditions, including reclaimed areas of oil shale opencast mining and semicoke hills.

Silver birch (*Betula pendula* Roth.), black alder (*Alnus glutinosa* (L.) Gaertn.) and Scots pine (*Pinus sylvestris* L.) were the three species selected for study as perspective species for recultivation in alkaline wastelands. They exhibit different growth strategies; all species are early successional species and have also a good management prospects. The potential of black alder, silver birch and Scots pine for recultivation of exhausted areas of opencast oil shale mines and semicoke hills in relation to short-root adaptation is still poorly understood.

In this context, our main objectives were:

1) to evaluate the range of morphological parameters of short roots for the three main tree species (Scots pine, silver birch and black alder) used for recultivation of the areas of oil shale opencast mining and semicoke hills in Estonia;

2) to analyse the impact of soil conditions and stand age on the morphology of short roots. As a pilot project, the addition of phenol-degrading *Pseudomonas* strains to the rhizosphere of birch seedlings on semicoke hills and its impact on short-root morphology, as well as biomass distribution of seedlings, was included in our study.

Material and methods

Stand characteristics. The present study was carried out in five planted stands: 4-yr-old and 27-yr-old black alder (*Alnus glutinosa* (L.) Gaertn.), 4-yr-old and 27-yr-old Scots pine (*Pinus sylvestris* L.) and 4-yr-old silver birch (*Betula pendula* Roth) stands on reclaimed oil shale opencast mining spoil and Calcaric Regosols [21, 22] (Table 1). Four-yr-old stands were established in 2002 with 2-yr-old seedlings. Planting density used in different plantations was 5360 plants per ha for pine, and 2500 for alder and birch. In the 4-yr-old silver birch stand in the oil shale mining area pingo was found and some of the birch seedlings were eaten by game, decreasing the number of trees by more than a half (Table 1).

A pilot study was carried out in the area of semicoke hills; both bio-augmented and control plot (Table 1) were 10 m^2, and both were established in 2002 with 2-yr-old bare-root transplants of silver birch. Plant density was higher in the bioaugmented plot (Table 1).

Bioaugmentation experiment. The bioaugmentation experiment was carried out with a set of bacteria consisting of three strains isolated from a nearby polluted area. These three bacterial strains *Pseudomonas mendocina* PC1 (AF232713), *P. fluorescens* PC24 (AF228367) and *P. fluorescens* PC18 (AF228366) degrade phenols via catechol *meta*, catechol or protocatechuate

Table 1. **Characteristics of the studied alder, birch and Scots pine stands: tree species, location, site (reclaimed oil shale mining spoil, Calcaric Regosol [21, 22] and semicoke), age, number of tree stems per ha and soil characteristics: soil type, pH$_{KCl}$, Kjeldahl nitrogen (N, %), available (ammonium lactate soluble) phosphorus (P, mg kg^{-1})**

Stand/ location	Tree species	Trees (per ha)	Age, yr	Soil type		pH$_{KCl}$	N (%)	P (mg kg^{-1})
Sirgala 59º17'N 27º44'E	black alder	2300	27	Calcaric Regosol		7.0	0.55	43.7
	Scots pine	2670	27	Calcaric Regosol	O-horizon	6.7	0.98	97.6
					Mineral layer	8.2	0.13	23.8
Narva 59º18'N 27º46'E	black alder	2090	4	Oil shale mining spoil		7.5	0.04	43.6
	Scots pine	4060	4	Oil shale mining spoil		7.6	0.03	65.2
	silver birch	1170	4	Oil shale mining spoil		7.5	0.04	68.2
Kohtla-Järve 59º 23'N 27º13' E	silver birch control	4*	4	Semicoke		8.0	0.10	6.6
	silver birch bioaugmented	8*	4	Semicoke		8.6	0.08	4.7

* trees per 10 m^2

ortho or via a combination of catechol *meta* and protocatechuate *ortho* pathways, respectively [23]. In bioaugmentation experiments the biomass of these bacteria was supplied to the denser experimental plot (10 m^2) in June 2004. The treatment received 20 L of bacterial suspension with concentration 10^8 CFU ml^{-1}. The ratio of bacterial strains PC1, PC18 and PC24 in the suspension was 3:1:1.

Root sampling. In 27-yr-old black alder and Scots pine plantations the short-root sampling was carried out before budbreak (April), in mid-summer (June-July), and after fall (November) 2004. Root sampling from three 4-yr-old birch, alder and pine plantations on reclaimed areas of opencast oil shale mining and from two birch plantations in the areas of semicoke hills were carried out in November 2004. We collected ten root samples per stand in 27-yr-old stands by spading from the 10-cm-deep topsoil in random points, and in younger stands by excavating approximately one third of the root system of ten randomly selected trees. Two or three random subsamples were taken from each sample. In the frame of a pilot study in the areas of semicoke hills, three trees were excavated from control and three from bioaugmented areas; thereafter, five random subsamples were takes from each root system.

In the 27-yr-old pine plantation on the mineral soil layer a 1–3 cm thick poorly decomposed O-horizon had formed, mainly needle litter, which was densely rooted; short-root parameters were determined separately for O-horizon and 0–10 cm mineral soil layer, due to big differences in soil properties (Table 1).

The short-root samples were washed with tap water to remove soil particles and examined under a microscope [12]. Root samples were analysed by WinRHIZO^{TM} Pro 2003b [24]. The roots were dried at 70 °C for 2 h to constant weight, and were weighed.

Short root morphological characteristics: mean diameter (*D*, mm) and mean length (L, mm) of the root tip were determined using WinRHIZO; for mean volume (V, mm^3) of the root tip, we first calculated the volume of short roots in the sample (V_{sample}, mm^3):

$$V_{sample} = 0.25\pi D^2 \sum_{i=1}^{n} L_i \ , \tag{1}$$

where L = root length, D = root diameter and n = the number of short-root tips and then divided V_{sample} with the number (n) of root tips in the sample:

$$V_{tip} = \frac{V_{sample}}{n} \tag{2}$$

Root tissue density (RTD, kg m^{-3}), specific root area (SRA, m^2 kg^{-1}) and specific root length (SRL, m g^{-1}) were calculated as follows:

$$RTD=\frac{W_{sample}}{V_{sample}}, \tag{3}$$

$$SRA=\frac{S_{sample}}{W_{sample}}, \tag{4}$$

$$SRL=\frac{L_{sample}}{W_{sample}}, \tag{5}$$

where W_{sample}, V_{sample}, S_{sample} and L_{sample} are the dry weight, the volume of short roots, the surface area and the length of short roots in a sample (measured by WinRHIZO, except V, which was calculated as described above).

Root tip frequency (RTF; n mg d w^{-1}) was expressed as a tip number on a dry mass basis.

The biomass distribution of 4-year-old birches on semicoke hills. In the areas of semicoke hills, three trees were excavated from the control and three trees from the bioaugmented plot and the biomass allocation in the above- and belowground parts was studied. Root system was divided into fine (< 2 mm) and thicker fractions (≥ 2 mm).

Chemical analysis. Soil nitrogen content was determined according to Kjeldahl, Tecator ASN 3313. Determination of available phosphorus in the soil was performed by flow injection analysis (ammonium lactate extractable), with the use of Tecator ASTN 9/84; pH_{KCl} of samples was measured (Table 1). Analyses were performed at the Biochemistry Laboratory of the Estonian University of Life Sciences.

Statistical analysis. The normality of variables was checked by Lilliefors and Shapiro-Wilk's tests. Except for the tip weight (W), the root parameters were normally distributed. To normalize the W values, the log and square-root transformation were used. For all the parameters multiple comparison of means was applied using Tukey test for unequal n. Software STATISTICA 7.0 was used, significance level $\alpha = 0.05$ was accepted in all cases. Data set of short-root parameters was analyzed using principal component analysis (PCA) based on correlation matrix (with the computer program ADE-4 [25]). Prior PCA analysis short-root parameters values were log-transformed.

Results

Morphological adaptations of short roots in Scots pine, silver birch and black alder

All short-root tips of pine and >95% of birch and alder root tips were ectomycorrhizal. The short-root morphological characteristics based on direct measurements (D, W, L, V) had different variation patterns compared to functional characteristics (SRA, SRL, RTD; Table 2, Fig. 1). Mean diameter of short roots in a 27-yr-old pine stand (Sirgala) was significantly higher compared to mean short-root diameter in a 4-yr-old pine stand (Narva) and compared to deciduous species: alder and birch in all investigated stands. Mean diameters of studied species were in the following order: $D_{pine} > D_{alder} > D_{birch}$. Mean length of root tip was highest for black alder; pine and birch root tip lengths were similar as were the mean volume of short-root tips of both species. The mean weight of short-root tip was smallest for birch and did not differ between pine and alder (Table 2).

Derived functional characteristics – SRA, SRL and RTD – differed significantly among coniferous and deciduous tree species, with lowest SRA and SRL values and highest RTD values being found in Scots pine (Figs 1, 2). Comparing deciduous tree species, much higher SRA and SRL values (194 ± 6 m^2 kg^{-1} and 204 ± 26 m g^{-1}) occurred for silver birch short roots.

Table 2. **Mean short-root morphological characteristics (± standard errors) in Scots pine, black alder and silver birch stands. Different letters indicate significant differences between means (Tukey test, $p < 0.05$)**

Species	Stand	SRA m^2 kg^{-1}	SRL m g^{-1}	RTD kg m^{-3}	Diameter mm	Length mm	Weight mg	Volume mm^3
Scots pine	Sirgala	**67.9**^{a} *±4.4*	**48.3**^{a} *±5.2*	**140.5**^{c} *±7.5*	**0.46**^{c} *±0.01*	**1.5**^{a} *±0.1*	**0.031**^{b} *±0.002*	**0.23**^{ab} *±0.01*
	Narva	**64.1**^{a} *±6.1*	**51.5**^{a} *±7.3*	**162.5**^{c} *±10.5*	**0.40**^{b} *±0.01*	**1.8**^{a} *±0.1*	**0.036**^{b} *±0.002*	**0.22**^{a} *±0.02*
Black alder	Sirgala	**103.8**^{a} *±6.7*	**85.1**^{ab} *±8.0*	**106.7**^{b} *±11.4*	**0.39**^{b} *±0.01*	**2.6**^{b} *±0.3*	**0.030**^{b} *±0.003*	**0.32**^{b} *±0.03*
	Narva	**155.2**^{b} ± 6.0	**126.4**^{bc} *±7.1*	**69.6**^{a} *±10.2*	**0.39**^{b} *±0.01*	**4.1**^{c} *±0.2*	**0.034**^{b} *±0.002*	**0.50**^{c} *±0.03*
Silver birch	Narva	**193.9**^{b} *±6.1*	**195.5**^{d} *±7.3*	**66.6**^{a} *±10.5*	**0.33**^{a} *±0.01*	**1.7**^{a} *±0.2*	**0.010**^{a} *±0.002*	**0.15**^{a} *±0.02*
	Kohtla-Järve^{c}	**193.5**^{b} *±9.1*	**204.1**^{d} *±11.4*	**71.5**^{a} *±8.8*	**0.32**^{a} *±0.01*	**1.8**^{a} *±0.2*	**0.009**^{a} *±0.003*	**0.14**^{a} *±0.02*
	Kohtla-Järve^{b}	**156.9**^{b} *±9.1*	**152.6**^{cd} *±11.4*	**92.4**^{ab} *±8.8*	**0.34**^{ab} *±0.01*	**1.7**^{a} *±0.2*	**0.015**^{a} *±0.002*	**0.15**^{a} *±0.02*

^{b} bioaugmented plot, ^{c} control plot

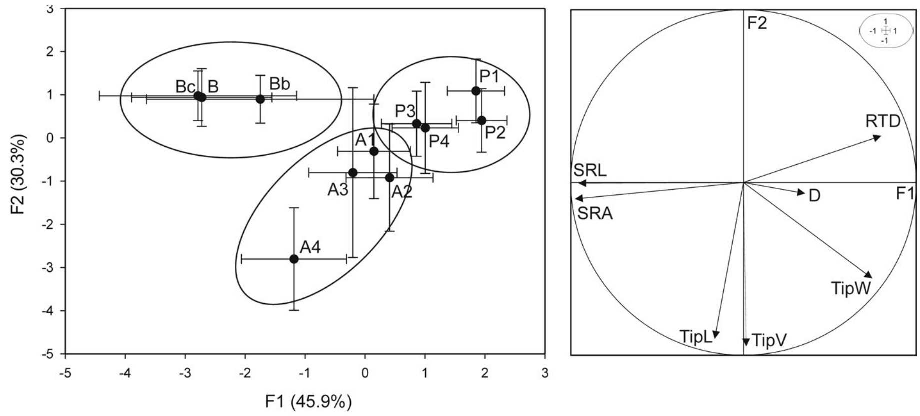

Fig. 1. Principal component analysis based on correlation matrix of short-root parameters. First and second principal components describe 45.9% and 30.3% of overall data variation, respectively. (A) Ordination of short root samples of different tree species along first two PCA axes (F1xF2). Shown are average values and standard deviations of sample scores for sampling locations by sampling time. Abbreviations: A1 – *Alnus glutinosa*, spring, Sirgala; A2 – *A. glut*., summer, Sirgala; A3 – *A. glut*., autumn, Sirgala; A4 – *A.glut*. autumn, Narva; B – *Betula pendula*, autumn, Narva; Bc – *B. pend*., autumn, Kohtla-Järve control plot; Bb – *B. pend*, autumn, Kohtla-Järve bioaugmented plot; P1 – *Pinus sylvestris*, spring, Sirgala; P2 – *P. sylv*., summer, Sirgala; P3 – *P. sylv*., autumn, Sirgala; P4 – *P. sylv*., autumn, Narva. (B) Correlation of short-root parameters with PCA axes

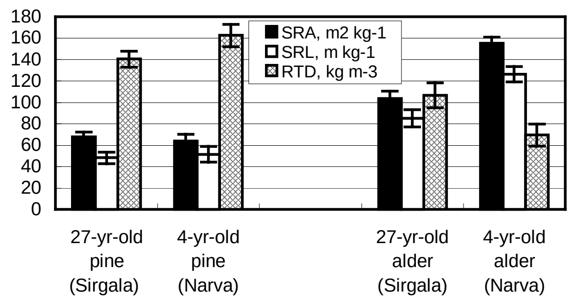

Fig. 2. Mean functional characteristics of Scots pine and black alder short roots (SRA, $m^2\ kg^{-1}$; SRL, $m\ kg^{-1}$; RTD, $kg\ m^3$) in 27-yr-old (Sirgala) and 4-yr-old (Narva) study areas. Bars indicate standard errors

Root tip frequency (RTF) was significantly higher for short roots of silver birch; short-root RTF of Scots pine and black alder were ~3.5 × lower. RTF is the inverse of mean mass of root tip, and it is not included in Table 2.

The results of principal component analysis for short-root characteristics (D, W, V, L, SRA, SRL and RTD) show that two axes account for 76% of the total variation of morphological parameters of short roots (Fig. 1). SRA, SRL, RTD and mean dry weight of root tip (W) were correlated with the first axis, and mean root tip length (L) and volume (V) with the second axis (Fig. 1).

Three groups differing in tree species were formed by principal component analysis (PCA) with respect to functional characteristics (Fig. 1).

Vertical pattern of short root parameters in topsoil in Scots pine plantations

Vertical distribution of fine roots in different layers was not estimated, but short-root parameters were determined separately for the O-horizon and for the mineral soil in summer (July) and autumn (November). SRA, SRL and mean diameter of short roots tended to be higher and RTD tended to be smaller in the O-horizon at both estimation times. However, the difference was insignificant in all cases. Mean weight of root tip was significantly smaller and RTF higher in the O-horizon in autumn; mean weight of tip was 0.028 ± 0.002 mg and RTF was 39.3 ± 2.8 in the O-horizon, compared to 0.035 ± 0.002 mg and 30.2 ± 1.5 n mg d w^{-1} in the mineral soil layer. Hence in later analysis, short-root data from O-horizon and mineral layer were bulked.

Impact of stand age on short root morphology

A comparison of short-root parameters between 4-yr-old and 27-yr-old alder stands in an oil shale mining area revealed significant differences; SRA and SRL of short roots were higher and RTD smaller in the younger black alder plantation (Fig. 2). Root tips in the younger black alder stand were $1.6 \times$ longer, however, mean diameter and mass of root tips in two different aged stands did not differ significantly (Table 2; Fig. 2).

The impact of stand age on short-root parameters in Scots pine was insignificant.

Biomass allocation and short root morphology of silver birch on semicoke

Both aboveground and belowground biomass of bioaugmented silver birch trees tended to be higher; still, the difference could not be proved, most probably due to small number of model trees. Relatively, the proportion of fine roots (< 2 mm) of total root biomass in bioaugmented trees was smaller than that of control birch trees.

SRA and SRL of short roots in bioaugmented birch trees tended to be smaller than those of short roots of birches from the control plot; the difference was insignificant. Only the RTD of bioaugmented silver birch short roots was significantly higher than that of same-aged silver birches planted on oil shale mining spoil (Fig. 3). The directly measured short-root parameters (D, W, L, V) did not vary between the different birch plots.

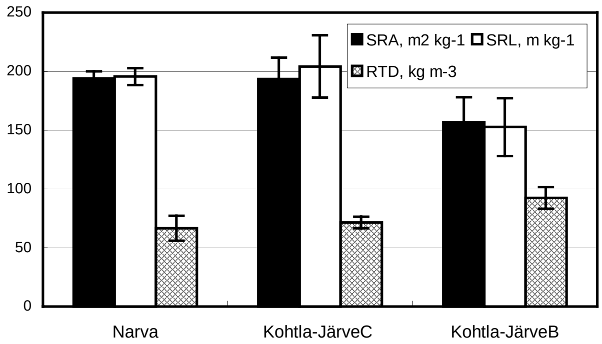

Fig. 3. Mean functional characteristics of silver birch short roots (SRA, $m^2\ kg^{-1}$; SRL, $m\ kg^{-1}$; RTD, $kg\ m^3$) in an oil-shale mining area (Narva) and in semi-coke areas (Kohtla-Järve, C-control plot, B-bioaugmented plot). The trees in all stands are 4-yr-old. Bars indicate standard errors

Seasonal dynamics of short-root characteristics in pine and alder plantations

In 27-yr-old black alder and Scots pine plantations, seven short-root characteristics were estimated before budbreak (April), in mid-summer (June-July), and after fall (November) to study seasonal dynamics of root parameters. All characteristics, except mean length of root tips for both tree species and SRL of alder short roots, varied significantly during the growing season (Fig. 4).

Mean weight and volume of short-root tips decreased significantly for both tree species in autumn; simultaneously, RTF of alder and pine increased significantly, therefore a lot of new short-root tips were formed. Moreover, mean diameter and SRA were also higher and RTD smaller for both species at the end of the vegetation period in autumn. Mean D, SRA and RTD values varied seasonally for alder short roots from 0.29 to 0.39 mm, from 79 to 104 $m^2\ kg^{-1}$ and from 107 to 180 $kg\ m^{-3}$ (Fig. 4BD), respectively. Mean D, SRA and RTD values varied seasonally for pine short roots from 0.33 to 0.46 mm, from 43 to 68 $m^2\ kg^{-1}$ and from 141 to 289 $kg\ m^{-3}$, respectively (Fig. 4AC). The species-related differences in short root size were stable during the vegetation period.

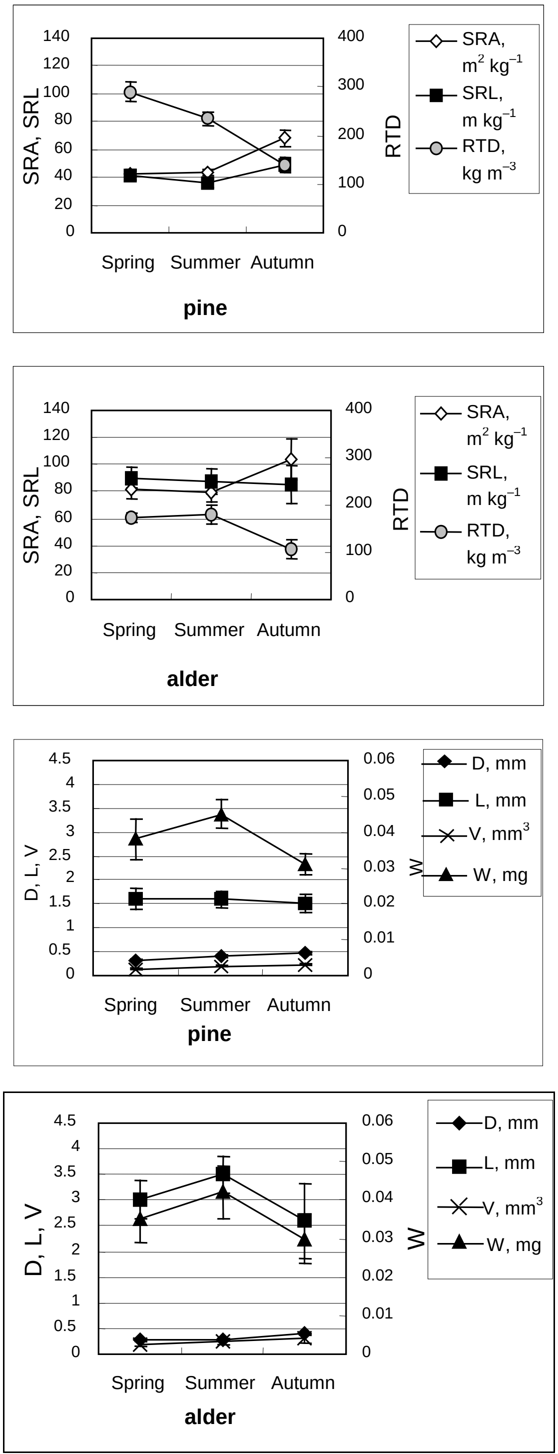

Figure 4. Seasonal dynamics of short-root parameters of black alder and Scots pine in 27-yr-old stands in an oil shale mining area. Bars indicate 95 % confidence limits.

Discussion

Our results indicate different strategies of short-root morphological adaptations in conifer (Scots pine) and deciduous tree species (black alder and silver birch) in oil shale mining areas. The short roots of Scots pine were relatively thicker (Table 2) and their SRA and SRL were 1.5–2.5 times lower than those of deciduous tree species; the difference was higher in 4-yr-old stands (Figs 1, 2). The means of short root SRA varied from 43 to 68 $m^2\ kg^{-1}$ for pine and from 79 to 155 $m^2\ kg^{-1}$ for alder during the whole growing season and for stands of different age (Fig. 4 and Table 2). Specific root area of Norway spruce ECM has been recorded to vary from 28 to 63 m^2 per kg dry weight [11, 12, 26–29]. However, SRA and SRL tend to be smaller and RTD higher for coniferous tree species. The mean RTD for Scots pine in this study varied from 141 to 163 $kg\ m^{-3}$, seasonally from 141 to 289 $kg\ m^{-3}$, and mean RTD for Norway spruce in our previous study varied from 190 to 540 $kg\ m^{-3}$ [12]. Mean RTD values for deciduous trees, silver birch and black alder in this study varied on a smaller scale, from 67 (Narva birch stand, autumn) to 180 $kg\ m^{-3}$ (Sirgala alder stand, summer). Bauhus and Messier [30] analysed soil exploitation strategies of fine roots in different tree species and concluded that conifers appear to follow a conservative strategy, since they have a relatively “coarse” and presumably long-living fine-root system. Silver birch and black alder are early-successional and stress-tolerant species [5], and higher SRA and/or SRL values in our study indicate a larger surface area per root dry weight or length. Hence, the larger is the SRA, the more effective is the economic strategy of allocation of assimilates to the build-up of short roots. Mean SRL of short roots varies similarly to SRA, since, as SRA is inversely related to RTD and D, then SRL is inversely related to RTD and D^2. Hence, the impact of root diameter is more essential for SRL.

Change in short-root morphological characteristics is a way for plants to adapt to environmental conditions, but different tree species have their own strategy for better mineral nutrition. Total variation of short-root morphological characteristics includes a genetically-determined component and the changes that are most probably caused by abiotic and biotic environmental factors. Short-root morphological measurements related to their size were dependent on tree species, but, among biotic factors, mycorrhizal short roots are influenced by the fungal symbiont, and the effect of that is not excluded. Although the short roots of pine were all mycorrhizal, and over 95% of the investigated short roots of alder and birch were mycorrhizal, the fungal species forming the mycorrhiza may vary significantly depending on the tree species.

The effect of tree age, 4 years in Narva and 27 years in Sirgala black alder stand at the time of morphological analysis, was significant (Table 2, Fig. 2). The SRA and SRL were significantly smaller in the middle-age black alder stand. A comparison of short root characteristics in the 4-yr-old pine stand and in the 27-yr-old pole pine stand did not reveal any statistically significant differences; further investigation is needed to elucidate whether the impact of tree age is significant in Scots pine. In our study only 4-yr-old birch stands were included, but, comparing the values of root characteristics with short-root parameters of silver birch in an older (80 year) natural mixed forest on calcareous soil (pH = 7.9; [12]), we can conclude that tree age has an impact on short-root parameters in birch stands. The SRA and RTD were 194 m^2 kg^{-1} and 67 kg m^{-3} for the 4-yr-old birch stand in the oil shale mining area and 84 m^2 kg^{-1} and 156 kg m^{-3} for mature silver birches in calcareous soil. Higher SRL values at younger tree age were revealed for two deciduous species, *Quercus alba* and *Acer saccharum*, where SRL of roots of first and second orders was approximately two times higher for one-year-old seedlings [31] than for older trees [16]. Higher SRA and smaller RTD in younger plantations could be explained by a bigger proportion of younger roots in the short-root population. Although, silver birch and black alder belong both to the same family *Betulacea*, the short-root parameters were significantly different between the two species in young stands (Figs 2 and 3, Table 2).

The initial site conditions on reclaimed oil-shale mining spoil in Sirgala and Narva were similar before planting of different tree species. However, soil conditions were changed within initial pedogenesis [21, 22] in the 27-year-old black alder and Scots pine plantation (Table 1). In the pine stand an O-horizon had formed, which consisted mainly of poorly decomposed pine needle litter; conditions in this layer differed from those in the mineral soil (e. g. pH was reduced; Table 1). Short-root SRA, SRL, D and RTF of pine tended to be higher and RTD and mean weight of root tip smaller in the organic horizon. Leuschner et al. [9] reported that SRA of fine roots of *Fagus sylvatica* increased in upper horizons at the expense of a reduction in D. In our case both SRA and D of pine were larger in the O-horizon. However, in our study the SRA and RTF of Scots pine short roots showed a similar pattern among the soil horizons as for some other tree species [9], with lower SRA values being found in lower horizons of a soil profile. Hence, pine litter from above- and belowground and roots with accompanying microbes and ectomycorrhizal fungi create and maintain their own milieu by actively transforming biotic and abiotic components of the calcareous opencast mining spoil in upper horizons.

Seasonal dynamics of short-root characteristics in 27-yr-old black alder and Scots pine plantations had similar tendencies for both tree species. SRA was higher and RTD smaller in autumn, after fall. Root tip frequency was significantly higher for both tree species in autumn, when mean weight of the root tip and RTD were the smallest. This appears to be due to the new generation of young roots formed in autumn. High SRL is found in young root systems [18], and RTD has been found to be smaller for young unsenescent roots [14].

The large SRA and SRL values in autumn may have been affected by the large amount of precipitation during the previous growing season in 2004.

Craine and Lee [32] have shown that in wetter sites the roots of herbaceous species have a smaller tissue density.

The effects of the composition and properties of the rhizosphere microbial community on the growth of seedlings are already clearly detectable in the one-year pilot project area of semicoke hills. Silver birch trees bioaugmented during one vegetation period by three phenol-degrading strains of *Pseudomonas* tended to have higher biomass above ground as well as below ground. Relatively smaller proportion of <2 mm root biomass of the total root biomass may indicate better adaptation of root system in bioaugmented treatment. The significantly larger RTD of bioaugmented birch short roots compared to that of the birch seedlings planted on oil shale mining spoil may indicate the lengthening of the life span of short roots on bioaugmented semicoke.

Accordingly to our results, it can be concluded that morphological adaptation of short roots of trees to corresponding soil conditions is specific for tree species.

The impact of tree age on short-root parameters was significant for both deciduous tree species; SRA and SRL were lower and RTD was higher in middle-aged black alder and mature silver birch stands. It needs further investigation to elucidate whether the impact of tree age is significant in Scots pine.

Root parameters on the areas of semicoke hills depend on the microbial community, as proved by the bioaugmentation experiment, but further investigation is needed to discover how the microbial community affects the rhizosphere and root morphology. Two mechanisms may be involved: reduction of semicoke toxicity to plants due to addition of bacterial biomass or direct impact of added microbial strains in the way similar to plant growth promoting bacteria.

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