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Tree age effect on fine-root and leaf morphology in a silver birch forest chronosequence

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Abstract The influence of forest ageing on fine-root morphology and relations between fine-root and leaf characteristics is poorly studied. The aim of this study was to analyse age-driven changes in ectomycorrhizal roots (EcM roots) and leaf morphology in a chronosequence of silver birch (*Betula pendula* Roth.), which would provide a better understanding of adaptation responses and acclimation capacity of tree roots and leaves. The chronosequence included six age classes (3, 6, 14, 32, 45, and 60 years.). All stands had regenerated naturally and grew in a highly productive *Oxalis* forest site type in Estonia. Most changes in the morphology of EcM roots and leaves of silver birch occur faster at a young age. The functional parameters— mean specific area of EcM root (SRA) and leaf specific area (SLA) as well as leaf N—decreased with age. EcM root SRA and specific root length (SRL) decreased with stand age as a result of increased mean diameter and tissue density. In age classes of 6, 14, and 32 years, the total number of dominating EcM taxa was 34, and the distribution of four different dominating EcM exploration types (contact-, short-, medium-, long-distance) was similar. We conclude that high values of SRA, SLA, and leaf N measured in young silver birch stands indicate high activity of physiological processes necessary for fast-growing young trees. A decrease of SLA and SRA and N in the chronosequence of fertile stands of silver birch is most probably caused by down-regulation of growth, affecting simultaneously leaves and fine roots.

Keywords Ageing · Betula pendula · Ectomycorrhizal roots · Leaf Morphology · Mineral nutrition · Root morphology

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Introduction

Fine roots, especially ectomycorrhizal (EcM) root tips, and leaves are key units for resource acquisition in ectomycorrhizal tree physiology: ectomycorrhizas with their external mycelia for nutrient uptake, and leaves for C transfer from the atmosphere (Helmisaari et al. 2009). To provide sufficient nutrient uptake, fine roots display morphologic plasticity by selectively allocating biomass or/and physiological plasticity by alterations in uptake rate per unit root length and unit area (Hodge et al. 2009; Richardson et al. 2009). Many studies have investigated the effect of environmental conditions on the fine-root morphology of trees, mainly for coniferous tree species (Bakker et al. 2009; Borken et al. 2007; Helmisaari et al. 2009; Konôpka and Takáčová 2010; review of Ostonen et al. 2007a) but also for deciduous species (Leuschner et al. 2009; Makita et al. 2011; Richter et al. 2007; Rosenvald et al. 2011a). Fewer studies have dealt with the influence of forest ageing on fine-root morphology and the

functional role of root adaptations and acclimations (Børja et al. 2008; Jagodziński and Kałucka 2010; Lõhmus et al. 2007; Rosenvald et al. 2011b). Stand-age-related variation in fine- and EcM root morphology is important for understanding complex ecophysiological changes during stand development.

The specific root area (SRA) is a measure of the water and nutrients absorbing surface area of roots per unit mass; specific root length (SRL) is a parameter of the ability of roots to proliferate in the soil (Bauhus and Messier 1999) and an indicator of the efficiency of capturing P (Richardson et al. 2009). SRA and SRL are both related to root efficiency because it has been reported that resource acquisition is proportional to length or surface area, and root (construction and maintenance) costs are proportional to mass (Eissenstat and Yanai 1997; Lõhmus et al. 1989; Ostonen et al. 2007a). Morphological parameters of ectomycorrhizal roots (EcM roots) reflect different fine-root acclimation and adaptation strategies for ectomycorrhizal trees, because these roots are a short-lived and continuously renewable functional compartment of the fine-root system (Guo et al. 2004; Ostonen et al. 2007b, 2011; Pregitzer et al. 2002).

First- and second-order silver birch roots with intact cortex are, as a rule, ectomycorrhizal (Smith and Read 1997; Taylor et al. 2000). The colonising EcM has a significant impact on root tip morphology (Comas and Eissenstat 2009; Ostonen et al. 2009; Sun et al. 2010). Growth of EcM hyphae can greatly enhance the absorption capacity of the root system (Rousseau et al. 1994). As EcM hyphae cover nearly all nutrient absorbing regions in roots (Taylor and Peterson 2005), and individual EcM fungal species have different nutrient uptake (solubilisation, assimilation, and transfer) capacities (Courty et al. 2010), colonising EcM community may have significant consequences for host plant nutrition. Agerer (2001) classified EcM into exploration types according to distinct foraging strategy and suggested that exploration types may help to categorise EcM with respect to their nutrient uptake efficiency. Relation between EcM, root morphology, and exploration type of colonising fungi was determined by Ostonen et al. (2011) for Norway spruce.

Trees exhibit plasticity in leaf morphology, allowing them to optimise photosynthetic efficiency as well as other ecophysiological functions (He et al. 2008; Lambers et al. 2006). Specific leaf area (SLA) describes the transpiring/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 utilisation (Lambers et al. 2006). Studies on deciduous species report the decrease of SLA with increasing tree age (Mediavilla and Escudero 2009) and/or tree size (Niklas

and Cobb 2008, 2010; Sellin and Kupper 2006; Thomas 2010). Leaves and EcM roots are physiologically the most active and functionally homogeneous parts of ectomycorrhizal trees. Although there is evidence that root morphological traits are driven by leaf traits (Comas and Eissenstat 2004; Withington et al. 2006), still very few studies have analysed EcM root trait–leaf trait relationships. Hence, the relations between leaf and root traits during ontogenesis are poorly understood as well.

In Northern Europe, birch (*Betula* sp.) is commercially the most important broadleaved tree species (Hynynen et al. 2010). This fast-growing pioneer species regenerates profusely after disturbances (clear-cut, fire) on open ground in fertile sites (Hynynen et al. 2010). Dense rooting, high fine-root biomass, and high foraging capacity (Curt and Prevosto 2003; Priha et al. 1999), as well as fast growth make silver birches (*Betula pendula* Roth.) competitive as a pioneer tree species (Hynynen et al. 2010). Fine roots including EcM roots of silver birch show higher morphological plasticity than fine roots of black alder, Norway spruce, or Scots pine (Kalliokoski et al. 2010; Lõhmus et al. 2007).

SRA and SRL of silver birch EcM roots have been shown to decrease, and tissue density and diameter to increase with age in plantations in an abandoned oil shale mining area, where growth conditions are harsh, especially initially (Rosenvald et al. 2011b). In this paper, we aim to analyse the ontogenetic changes in silver birch EcM root and leaf morphology in a fertile forest site type by using a chronosequence of silver birch. A novel approach of this study is the ascertaining of relations between ontogenetic changes in EcM root and leaf morphology and the exploration types of the colonising EcM. EcM root morphology, leaf SLA, leaf NPK nutrition status, and soil conditions were measured, and the dominating EcM morphotypes and their exploration types were detected in silver birch stands of different ages growing in the *Oxalis* site type. Hypotheses: (1) Functional parameters for tree mineral nutrition—EcM root SRA and SRL—decrease with tree age in a chronosequence of fertile silver birch forests; (2) changes in leaf and EcM root performance are related and coordinated—physiological activity and efficiency indicators: specific leaf area, EcM root area, and leaf N% all decrease with tree age; (3) the exploration type of colonising fungi affects EcM root morphology of silver birch.

Materials and methods

Description of study sites

We studied five silver birch stands: mature (60 years), maturing (45 years), middle-aged (32 years), young

Table 1 Stand and soil characteristics (mean ± SE)

Stand	Age class	Stand characteristics			Soil characteristics			
		H (m)	D (cm)	Trees/ha	Soil type	pH$_{KCl}$	N (%)	C/N
Järvselja	3	n.e.	0.72*	100,000	Umbric Gleysol	3.0	0.871 ± 0.104	21.6
	6	1.3	3.4					
Alatskivi I	14	9.9	11.5	1,350	Umbrisol	3.7	0.253 ± 0.037	13.9
Alatskivi II	32	10.8	17.0	3,210	Endogleyic Umbrisol	3.9	0.226 ± 0.007	13.6
Erastvere	45	20.1	25.6	940	Albeluvisol	3.9	0.117 ± 0.001	18.6
Aakre	60	31.0	30.1	305	Umbrisol	3.8	0.157 ± 0.020	16.3

n.e.—not estimated, *—tree diameter at root collar

(14 years), and a clear-cut, where we sampled two age classes (3 and 6 years)—6 age classes altogether (Table 1). All studied stands had regenerated naturally and grew on a flat landscape in *Oxalis* site type in south-eastern Estonia. Estonia is situated in the hemiboreal vegetation zone (Ahti et al. 1968), within a transition zone from maritime to continental climate. The annual average precipitation varies between 550 and 800 mm, and the annual average temperature in Estonia is between 4.3 °C and 6.5 °C. In *Oxalis* site type, stands are highly productive; acidic soils are mostly well drained and generally have a thin organic (O) horizon and a low C:N (14…20), indicating rapid litter decomposition. (Lõhmus 1984). Selected site characteristics are presented in Table 1. All studied silver birch stands had closed canopies and a high proportion of silver birch (<90 %). The 14-year-old stand had been thinned 4 years earlier. In the 60-year-old stand, storm-damaged trees were removed during the period 2003–2008.

EcM root tip sampling, processing, and analysis

Root samples taken in October 2009 were used for all age classes, except for the 60-year-old stand, for which only root data taken in October 2008 were available. To detect the possible effects of year-to-year differences in meteorological conditions on EcM root morphology, we compared our 2009 data about three age classes (15, 32, and 45) to comparable data sampled in 2008.

For each age class, ten soil samples (20 × 20, to 20 cm depth) were randomly collected using a flat spade. The distance between sampling points was at least 3 m, covering the whole study site. The root fraction was cleaned of adhering soil by careful rinsing in tap water and, if necessary, with a small soft brush. Silver birch EcM roots include only first- and second-order roots. To characterise the morphology of EcM roots of each age class, two random EcM root subsamples (20–24 living EcM root tips) were taken per sample, altogether 356–480 EcM roots per stand. Mean diameter, length, and surface area of EcM roots were measured with WinRHIZO™ Pro 2003b for

each subsample. The air-dry EcM roots were dried at 70 °C for 2 h to constant mass and weighed to an accuracy of 0.01 mg. The following morphological parameters were measured or calculated (calculation is briefly described in Ostonen et al. 1999) for every age class: mean EcM diameter (D), length (L), mass (M), branching frequency (number of tips per root dry mass unit, RTF$_M$), tissue density (root mass divided by root volume, RTD), specific root area, and length (SRA and SRL).

Dominant morphotypes were identified, and morphological parameters of EcM roots were measured by morphotype for the three young (6-, 14-, and 32-year-old) stands of the chronosequence, because an earlier study (Rosenvald et al. 2011b) has shown that the fastest changes in EcM root morphology occur during the early development of silver birch stands. 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 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. Random EcM root subsamples for morphological studies were taken from each morphotype sample. Three individual root tips of each morphotype per sample were mounted into CTAB lysis buffer (100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2 % cetyl-trimethylammonium-bromide), maintained at room temperature until molecular analyses and subsequently identified using sequence analysis of the nuclear rDNA Internal Transcriber Spacer (ITS) region. The DNA was extracted using a Qiagen DNeasy 96 Plant Kit according to the manufacturer's instructions. Primers, PCR conditions, product purification, sequencing, and sequence processing are described in Tedersoo et al. (2010a). Sequences were assigned to species based on a 97.0 % ITS barcoding threshold (Tedersoo et al. 2003; except 99.0 % was used for *Cortinariaceae* and *Hydnangiaceae* that display little divergence in the ITS region). For species-level

identification, representative sequences of each species were subjected to a bulk megablast search against International Nucleotide Sequence Databases (INSD) as implemented in the PlutoF workbench of the UNITE database (Abarenkov et al. 2010). Identified morphotypes were divided into four exploration types according to Agerer (2001), and the exploration types were ranked as follows: contact- (1), short- (2), medium- (3), and long-distance (4). The not colonised roots formed the fifth group.

Total N and C concentrations of EcM roots were determined using CHN analyser PerkinElmer 6400 Series II in the laboratory of the Department of Geology of the University of Tartu.

Soil and leaf sampling and analysis

Soil samples of a stand were taken from 10 points (same points as root samples) in October 2009 for all stands except for the 60-year stand, where samples were taken in October 2008. Soil nitrogen was determined by the Kjeldahl method (Tecator ASN 3313). Determination of available (ammonium–lactate extractable) phosphorus in the soil was performed by flow injection analysis (Tecator ASTN 9/84). Organic matter was determined by loss of ignition at 360 °C; pH_{KCl} was measured.

Model trees were felled in August 2008 and 2009, when the process of annual leaf biomass formation was complete, and processed according to Uri et al. 2007. The trees of each sample plot were divided into five classes on the basis of $D_{1.3}$. Model trees were taken according to the received frequency distribution, 6 (in 45-, and 60-year-old stands), 5 (in 32-year-old stands), or 12 (in younger stands) trees per stand. In all cases, the model trees were felled in the middle of the sample plot to avoid edge effects. The crowns of the model trees were divided into five or ten sections depending on the size of the tree, except the crowns of the youngest trees (6 years), which belonged fully to one section. The concentrations of leaf NPK and SLA of 5–6 model trees were calculated as the weighted averages, considering the share of a particular section in the tree leaf biomass. For SLA estimation, 20–25 leaves were randomly taken from each crown layer of a model tree and dried under pressure. Leaf area (including the petiole) of each leaf was measured using the program WinFOLIA (Regent Instruments, Inc.). Measured leaves were dried to constant mass; each leaf was weighed to an accuracy of 0.1 mg, and specific leaf area (SLA) was calculated. Leaf nitrogen concentration was determined by the block digestion and steam distillation methods (Tecator AN 300). To measure leaf phosphorus concentration, digest by flow injection analysis (Tecator AN 5242) and the analyser Fiastar 5000 (ISO/FDIS 15681) was used. Leaf potassium content was determined by flame photometry (Method 956.01), using

Sherwood Model 425 Flame Photometer. Analyses were performed at the Laboratory of Biochemistry of the Estonian University of Life Sciences.

Statistical analyses

Data were tested for normal distribution using a Shapiro–Wilk and Lillefors test. To obtain normal distribution, EcM root length and SRL were log-transformed. Differences between annual means in an age class were evaluated by the t-test. Multiple regression analysis was used to find and estimate the effects of age and site properties (leaf and soil chemical attributes) on studied EcM morphological parameters. A partial correlation analysis was used to find relations between EcM root morphology and site properties after removing the influence of age. Variation of a morphological parameter in the chronosequence was evaluated by the coefficient of variation of stand means. Gamma correlation coefficients (Gamma r) were calculated to examine relationships between EcM root morphological parameters and exploration-types ranks of EcM morphotypes. Significance level $\alpha = 0.05$ was accepted in all cases. Data analyses were performed using STATISTICA 7.1; CANOCO version 4.52 software was used for redundancy analysis (RDA) and to create an ordination plot of morphological parameters and sites. All means are presented with standard errors (mean $\pm$ SE).

Results

EcM root morphology and N concentration in the silver birch chronosequence

All studied EcM root morphological and architectural parameters (mean diameter and mass, branching frequency, specific root area and length, tissue density) were strongly dependent on tree age (Fig. 1a–f). Mean EcM root mass and tissue density increased linearly with tree age. Mean EcM root diameter increased, and SRL and RTF_M decreased logarithmically with tree age. SRA correlated highly with SRL ($r = 0.95$, $p < 0.01$) and also decreased logarithmically with tree age. Length ($\pm$ SE) of individual EcM root was longest in the 60-year-old stand (1.36 ± 0.08 mm), but was generally similar among younger stands, varying between 0.96 ± 0.04 and 1.07 ± 0.07 mm. Most EcM morphological parameters changed faster in younger age (≤ 6 years). In the RDA ordination plot of morphological parameters, the two first PCA axes described 28 % of variation, and the stands were mostly ordered along the first axis by stand age (Fig. 2). Stand age described 22 % of the variation of the studied EcM root morphological parameters according to RDA analysis. Mean CV% of EcM root

Fig. 1 Means of EcM root morphological parameters in a chronosequence of silver birch stands (*bars* indicate standard errors)
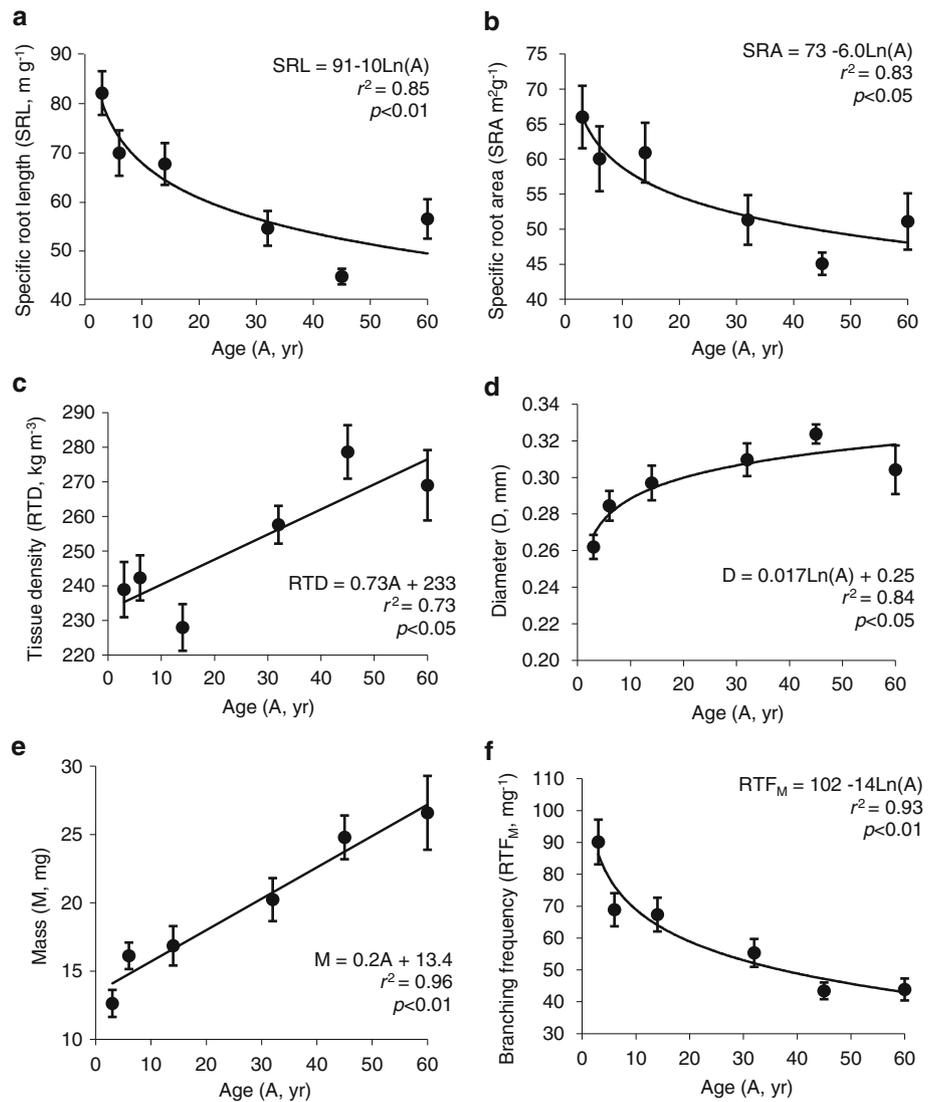
morphological parameters across the chronosequence ranged as follows: RTF_M (29), mass (28), SRL (21), SRA (14), length (13), tissue density (8), diameter (7).

In addition to tree age, leaf N was also an essential predictor variable in multiple regression analysis for the dependent variables: EcM root diameter, tissue density, and SRL (Table 2). Leaf N, as an indicator of tree N nutritional status, was positively related to EcM root diameter and tissue density and negatively to SRL. EcM root length, the only parameter not related to stand age, correlated negatively with leaf K%. Other correlations between EcM root morphological parameters and soil and leaf chemical properties were insignificant when age effect was excluded.

Between-year differences in EcM root morphological parameters were insignificant. However, there was a tendency for lower mean EcM root diameter and mass and higher tissue density in all studied stands in the drier year of 2009 (the amount of precipitation in the vegetation
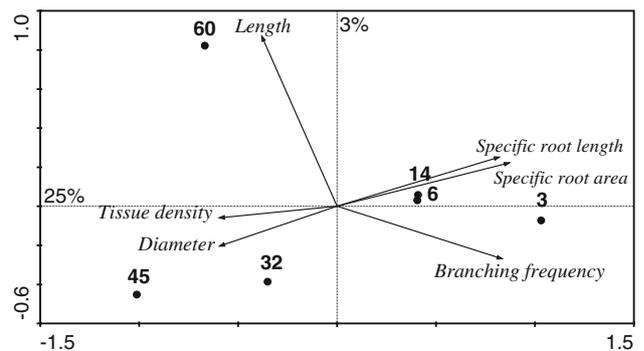

Fig. 2 Ordination plot of EcM root morphological parameters and stands of different ages (indicated by stand age)

period (April–September) was 16–22 % lower in 2009 compared to that in 2008).

EcM root N% varied across the chronosequence from 2.2 to 3.2 %; no stand-age-related trend nor significant

Table 2 Significant relationships between mean EcM root morphological variables, stand age and leaf nutrient concentrations according to regression analyses

Model	β	R^2 (%)	p
$SRL = 241 - 19.0\ln A - 53.5N_L$	$\beta_A = -1.7$, $\beta_{NL} = -0.9$	99	<0.001
$D = 0.015 + 0.030\ln A + 0.08N_L$	$\beta_A = 1.7$, $\beta_{NL} = 0.8$	96	<0.005
$RTD = -16 + 1.55A + 97N_L$	$\beta_A = 1.8$, $\beta_{NL} = 1.1$	92	<0.05
$L = 2.22 - 1.41K_L$	$\beta_{KL} = -0.96$	89	<0.005

SRL (m kg^{-1})—specific root length, D (mm)—diameter, RTD (m^2 kg^{-1})—tissue density, A—stand age (yrs), N_L—leaf N%, K_L—leaf K%, β—standardised regression coefficient of age (β_A), leaf N% (β_{NL}), and leaf K% (β_{KL}), R^2—coefficient of determination, p—level of probability. Insignificant ($p > 0.05$) intercepts are in *italics*
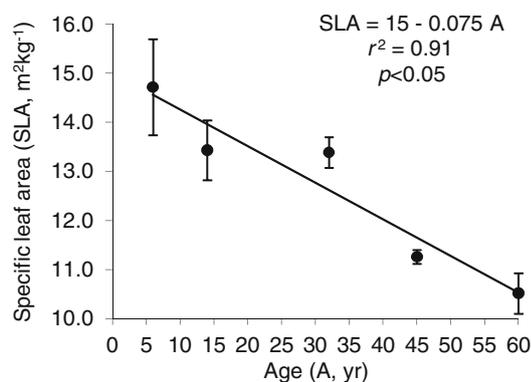
Fig. 3 Specific leaf area in the silver birch chronosequence (*bars* indicate standard errors)

correlations with morphological parameters of EcM roots were found. Root N% was higher than leaf N% across the chronosequence, except for the two youngest age classes.

Dynamics of leaf SLA and NPK% in the chronosequence

Specific leaf area (Fig. 3), leaf N% (Fig. 4a), and leaf K% (Fig. 4c) decreased with tree age. Leaf N% correlated positively with soil N% (Table 1, $r = 0.85$, $p < 0.05$). Leaf P nutrient status improved with age—leaf P% increased with stand age (Fig. 4b) and was positively correlated with soil pH ($r = 0.91$, $p < 0.05$; Fig. 2b), which increased likewise with age. Leaf P:N ratio was near to optimal in the three older stands (Fig. 4d).

Ecytomycorrhizal fungal morphotypes

Molecular analyses revealed 33 EcM fungal species among the dominant morphotypes in the three studied age classes (6, 14, 32 years; Table 3). EcM community structure of the dominant morphotypes differed among the age classes of the chronosequence. We found only three species and one morphotype (*Tomentella sublilacina*, *Tomentella badia*, *Cenococcum geophilum*, *morph2*) in more than one stand. Uncolonised roots were found dominating in only a few
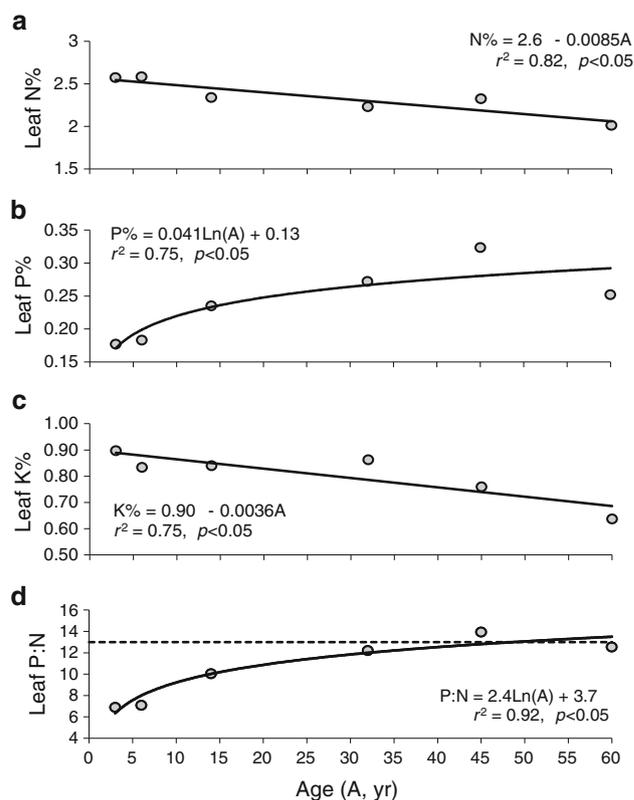
Fig. 4 Dynamics of leaf N, P, K concentrations (%) and P: N ratio. *Dotted line* indicates the optimal N:P ratio in birch leaves according to Ingestad and McDonald (1989)

samples in each stand. The number of dominating EcM fungal species or morphotypes was 9 in the 6-year-old stand, 11 in the 14-year-old stand, and 18 in the 32-year-old stand. The following lineages of fungal taxa (according to Tedersoo et al. 2010b) were most commonly detected as dominant colonisers in birch root tips: tomentella-thelephora, russula-lactarius, amanita, cortinarius, and tricholoma.

Dominating EcM root tip colonisers belonged most frequently to short- or medium-distance exploration types for all studied age classes. A weak negative correlation was found between the exploration type rank and EcM root tissue density and mass (Gamma $r = -0.32$ and Gamma

Table 3 Dominating EcM fungal species or morphotypes in a chronosequence of silver birch growing in *Oxalis* forest site type

Tree age	Dominating EcM species or morphotypes
6	**Cenococcum**, *Cortinarius sp*, *Cortinarius illibatus*, *Cortinarius anomalus*, *Piloderma lanatum*, *Thelephoracea*, *Tomentella sp*, **Tomentella**
	sublilacina, morph1
14	*Amanita sp*, *Amanita mortenii*, **Cenococcum**, *Cortinarius umbrinolens*, *Laccaria laccata*, *Pachyphloeus marroninus*, *Russula betularum*,
	Tomentella sp, **Tomentella badia**, *Tomentella lapida*, **morph2**
32	**Cenococcum**, *Hebeloma sp*, *Hydnum rufescens*, *Inocybe striata*, *Lactarius tabidus*, *Lactarius vietus*, *Russula gracilis*, *Sebacinaceae*, *Tarzetta catinus*, *Thelephoracea*, **Tomentella badia,** *Tomentella stuposa*, **Tomentella**
	sublilacina, *Tuber pacificum*, **morph2**, morph3, morph4, morph5

Bold indicates EcM root morphotypes which were found in more than one age class

$r = -0.30$, respectively; $p < 0.01$). Rank of exploration type correlated positively, but also weakly with SRA and RTF$_M$ (Gamma $r = 0.31$ and Gamma $r = 0.30$, respectively; $p < 0.01$). According to the RDA analysis, the EcM fungal partner described 73% of the variation in EcM root morphology of the three age classes.

Discussion

As all studied stands belong to fertile *Oxalis* forest site type, characterised by acidic soils, and therefore grow in similar site conditions, we assumed that the trends in EcM morphological parameters and in leaf characteristics reflect age-dependent changes. It was also proved by RDA analysis—stand age increased along the first axis in the RDA ordination plot of EcM root morphological parameters. Stand density decreases considerably in the studied chronosequence, but such a decline also occurs naturally in forests.

EcM root morphology and leaf SLA

The age trends for EcM root traits in the current study were similar to those observed in a silver birch chronosequence (1, 2, 3, 5, 29, 40 years) in plantations in an oil shale mining area, where calcareous mine spoil initially had high stoniness and low organic content, and a primary succession of forest soil occurred (Rosenvald et al. 2011b). In both, the present study and the mine spoil chronosequence, younger trees had higher EcM root SRL and SRA as a result of lower mean diameter and tissue density. Both EcM root diameter and tissue density increased with age, despite very different soil conditions. EcM roots, inherent to the heterorhizic fine-root system of silver birch, form a functionally homogeneous compartment in the fine-root system, differently from a fine-root compartment that includes both roots with primary structure (living cortex) as

well as roots of secondary structure. However, for fine roots less than one or two mm in diameter, SRL and SRA values are also reported to be higher for younger trees in the case of silver birch (Kalliokoski et al. 2010) and other tree species (Fujimaki et al. 2007; Jagodziński and Kałucka 2010).

Previous land use has a significant effect on the EcM root morphology of silver birch. Young (6- and 14-year-old) birches growing in fertile *Oxalis* type forests had higher root tissue density and lower SRA than 8–14-year-old birches on agricultural land (Rosenvald et al. 2011a) and 5-year-old birches in a reclaimed oil shale mining area (Rosenvald et al. 2011b). SRL of young birches in forests (69 ± 3 m g^{-1}) and on agricultural land (75 ± 2 m g^{-1}) was significantly lower than SRL in the mining areas (113 ± 9 m g^{-1}).

EcM root branching frequency (measured as tips per unit root dry mass) was especially high in the young (3 yr.) silver birch age class and then decreased logarithmically with age; the same result was found in the birch chronosequence in mining areas (Rosenvald et al. 2011b). Analogous results for the finest root fraction of fine roots ($d < 1$ mm) are shown in Børja et al. (2008), where branching frequency of roots of Norway spruce (*Picea abies* (L.) Karst) was two times higher in the 10-year-old stand than in the 30-, 60-, and 120-year-old stands.

Irrespective of site and tree species, young trees need to build-up their root system quickly. Therefore, both EcM and fine roots of young trees are small in diameter, have high SRL, SRA, and branching frequency, and low tissue density, and consequently tend to have low construction costs. Age-related increases of root tissue density and root diameter may be caused by lower root turnover rate of EcM roots (longer life-span) in older trees (Eissenstat and Yanai 1997; Withington et al. 2006). In young stands, EcM root tissue density and also root diameter are lower due to fast growth and expansion of the root system at a young age. 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.

In the present study, the fastest changes in EcM root morphology of silver birch occurred at a very young age (≤6 years). This accords again with our earlier observations in the birch stand chronosequence in the oil shale mining area (Rosenvald et al. 2011b) and is consistent with the results of Claus and George (2005), who studied fine-root SRL in stand chronosequences for three tree species (*Fagus sylvatica* L., *Picea abies* (L.) Karst., and *Quercus cerris* L.) in Europe.

The sensitivity of EcM root morphological parameters to stand age was different. RTF_M or its inverse M, responded most sensitively to stand age: they had the highest variation across the chronosequence, and their value changed approximately twofold in 60 years. The order of sensitivity of morphological parameters to stand age in this study, in relatively homogeneous soil conditions, was the same as we observed for birch chronosequences growing in a reclaimed oil shale mining area (Rosenvald et al. 2011b). Hence, we suggest that the stand age effect on silver birch morphology is stronger than the site properties effect.

In the present study, the trends of most EcM morphological parameters change after the tree age of 45 years, though differences between the means of ages 45 and 60 years are insignificant. A possible reason may be the ageing of trees and a decline in growth after that age. Hynynen et al. (2010) reported that growth of silver birch is vigorous until a stand age of 40–50 years, and after this, growth starts to decline. The slowing of height growth with 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).

The specific leaf area decreased in the studied forest chronosequence; 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 et al. 2008), the 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 maximised with high SLA there. Moreover, Pearcy and Sims (1994) proposed that thicker leaves (with lower SLA)

may confer resistance to stress that may be prevalent in high light conditions. In addition, leaves of older trees may be more vulnerable to water stress due to the increment of tree height (Table 1). Hence, older stands of silver birch possibly decreased SLA to cope with changed environmental conditions such as higher light and lower leaf water potential. Decreased specific leaf area is the main factor associated with the decline in the relative growth rate (RGR) (e.g. Imada et al. 2010; Lambers and Poorter 1992; Reich et al. 1998); consequently, EcM root SRA should also decrease when tree growth slows down. A decline in the relative growth rate for aboveground parts of birch stands was found in the studied chronosequence (Uri et al. 2012).

The high values of specific areas of EcM roots and leaves at a young age indicate fast growth and low constructions costs. Leaves and EcM roots are physiologically the most active part of trees; hence, their morphological adjustment to the decline in tree performance should be most clearly expressed. Our assumption that the dynamics of leaf and root specific areas in the birch chronosequence may be similar was clearly proven—both parameters decreased with tree age.

NPK nutrition

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). Generally, nitrogen limits plant growth in boreal and cold temperate forests. According to critical NPK concentration in birch leaves (Ingestad and McDonald 1989), leaf N% and P% were higher than their deficient levels across the studied chronosequence, and K deficiency was apparent in all age classes.

Leaf N% declined linearly with stand age. Our finding agrees with a birch study by Thomas (2010) where smaller *Betula alleghaniensis* Britt. trees had higher leaf N% and SLA than bigger ones. The decrease in leaf N concentration with tree age is also shown for evergreen *Eucalyptus grandis* W. Hill ex Maid. (Graciano et al. 2007) and for deciduous *Quercus acutissima* Carruth. (Noh et al. 2007). High leaf N at a young age, indicating high leaf photosynthesis (through an increase in the pool of enzymes and other proteins involved in light interception, light conversion, and carbon fixation) and growth and maintenance respiration (Delagrange 2011; Hassiotou et al. 2010; Reich et al. 1998; Cannell and Thornley 2000), is necessary for rapid growth in young birches competing strongly for light and other resources due to high stand density. The age-related decrease of leaf N concentration could be due to 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 et al. 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. In our study, the highest leaf N of the youngest trees may also be influenced by high soil N in young stands, where nitrogen mineralisation is high immediately after a disturbance (clear-cut) as a result of increased decomposition and decreased biological demand (White et al. 2004). Hence, leaf N declined simultaneously with SLA in the silver birch forest chronosequence.

In the current study, after removing the age effect, leaf N was negatively related to EcM root SRL and positively to diameter and tissue density. Despite the positive relation between leaf N and EcM root diameter and tissue density, leaf N decreased and EcM root diameter and tissue density increased with age. Hence, the effect of age on root morphology seemed to be the stronger than the effect of leaf N. In the case of lower N availability, EcM roots are thinner and have lower tissue density, resulting in high SRL and low root construction costs. This finding corroborates the findings of earlier studies about silver birch, which report a decrease in EcM and fine-root (d < 1 mm) SRL with site fertility (Kalliokoski et al. 2010; Rosenvald et al. 2011a); also meta-analyses in Ostonen et al. 2007b revealed that fine-root SRL decreased with fertilisation. High SRL and SRA could be the root nutrition stress indicators for silver birch, as reported in Rosenvald et al. (2011b). However, in contrast, in mature silver birch stands, EcM roots were found to have the highest SRA and SRL in the most fertile (*Oxalis*) forest site type when different site types and latitudinal locations were compared in a study by Ostonen et al. 2007a.

Leaf P concentration increased with age and with soil pH. Inorganic phosphorus availability to plants is strongly influenced by soil pH and is considered to be maximal when pH_{KCl} is between 5.5 and 6.5 or strongly alkaline (Lambers et al. 2006); however, opinions vary (Marschner 2005). In very acidic soils, as in our youngest stand ($pH_{KCl} = 3.0$), P is fixed to a harsh–soluble form of Fe- and Al-phosphates, and inorganic P-resources are limited. Leaf P% increased logarithmically with stand age also in silver birch plantations growing in a reclaimed oil shale mining area, where, during the first 7 years, leaf P increased from 0.19 ± 0.04 to 0.32 ± 0.04 %, and the soil pH decreased from 8.1 to 7.5 (Kuznetsova et al. 2010). Age-related changes in leaf P of deciduous species are very poorly studied and controversial. Leaf P decreased in the *Quercus acutissima* chronosequence (Noh et al. 2007); however, a positive relation between forest age and available soil P has been reported in an evergreen deciduous *Eucalyptus regnans* F. Muell. forest, where both

phosphatase activity and labile organic phosphorus increased with forest age (Polglase and Attiwill 1992).

Leaf K decreased with stand age, and limited growth across all age classes. K deficiency in all stands may be aggravated by leaching of K from acidic soils of the chronosequence; however, additional studies are needed to ascertain the reasons for low K.

EcM root N% was a relatively autonomous root parameter in the studied silver birch chronosequence—no age trend appeared, and EcM root N did not correlate with root morphological parameters nor with leaf N. When comparing 3-year-old birches, the EcM root N% measured in our study (2.17) was similar to that determined in the mining area (2.23), found in Rosenvald et al. 2011b; a similar range was reported for EcM root N% of 3-year-old *Quercus robur* L. (1.97 ± 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). EcM root nitrogen status was little dependent on tree age, most probably because EcM roots are first served by assimilated nutrients and achieve first the necessary N concentration. In the current study, root N% was higher than leaf N% across the chronosequence, except for the two youngest age classes. 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.

EcM species and morphotypes in the silver birch chronosequence

The effect of the EcM fungal partner on EcM root morphology was strong—according to the RDA analysis, colonising EcM species described 73 % of the morphological variation. Our study revealed 33 dominating EcM species or morphotypes colonising roots of silver birch in total in the studied three age classes. Twieg et al. (2007) found in total 82 EcM fungal species colonising paper birch in the chronosequence (5, 26, 65, and 100 years) 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 between all pairs of age classes, but were similar in 65- and 100-year-old stands. Despite the fact that different EcM root colonising species dominated in different age classes, distribution of EcM exploration types affecting the nutrient acquisition efficiency of EcM roots (Agerer 2001) was similar across the chronosequence in our study. Hence, we suppose that the effect of EcM community on EcM morphology of silver birches is similar across the chronosequence, and the observed differences in root morphological characteristics are driven mainly by the ontogenesis of the tree. Like in the case of Norway spruce (Ostonen et al. 2011), silver birch EcM roots colonised

with long exploration type forming fungi had higher SRA and lower tissue density than EcM roots colonised by other exploration types forming fungi. However, we cannot exclude the differences in nutrient acquisition efficiency of EcM roots within an exploration type. We also cannot distinguish the effects of site and tree age on a community of dominant EcM species and their exploration types; more studies are needed.

Conclusions

The most rapid changes in EcM root morphology of silver birch growing in naturally regenerated fertile stands occur at a young age, before 5 to 10 years. Our first hypothesis that EcM root SRL and SRA—functional parameters for tree mineral nutrition—decrease with increasing stand age was proven. The decrease was caused by increasing root tissue density and diameter. Both results: (1) exploration type of EcM fungi affects EcM morphology, and (2) colonising EcM species describe the major part of the morphological variation, support our third hypothesis that the EcM fungal partner shapes EcM root morphology. The coordination between leaf and root morphology and physiology (second hypothesis) was also confirmed—SRA and SLA decline simultaneously with leaf N in the birch chronosequence. High values of the mentioned parameters indicate fast growth (high RGR) and intensive physiological processes and allow young trees to grow rapidly. The similar tendencies for SLA and SRA are most probably caused by the down-regulation of growth, affecting simultaneously leaves and fine roots. When the effect of stand age was excluded, leaf N was negatively related to EcM root SRL and positively to root diameter and tissue density. Hence, the construction costs per EcM unit length should be lower in lower N supply.

References

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T et al (2010) The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytol 186:281–285

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Ahti T, Hamet-Ahti L, Jalas J (1968) Vegetation zones and their sections in northwestern Europe. Ann Bot Fenn 5:169–211

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

Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Can J For Res 29:260–273

Børja I, De Wit HA, Steffenrem A, Majdi H (2008) Stand age and fine root biomass, distribution and morphology in a Norway spruce chronosequence in southeast Norway. Tree Physiol 28:773–784

Borken W, Kossmann G, Matzner E (2007) Biomass, morphology and nutrient contents of fine roots in four Norway spruce stands. Plant Soil 292:79–93

Cannell MGR, Thornley JHM (2000) Modelling the components of plant respiration: some guiding principles. Ann Bot (London) 85:45–54

Claus A, George E (2005) Effect of stand age on fine-root biomass and biomass distribution in three European forest chronosequences. Can J For Res 35:1617–1625

Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Funct Ecol 18:388–397

Comas LH, Eissenstat DM (2009) Patterns in root trait variation among 25 co-existing north american forest species. New Phytol 182:919–928

Courty PE, Buée M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault M-P, Uroz S, Garbaye J (2010) The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. Soil Biol Biochem 42:679–698

Curt T, Prevosto B (2003) Rooting strategy of naturally regenerated beech in silver birch and Scots pine woodlands. Plant Soil 255:265–279

Delagrange S (2011) Light- and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. Environ Exp Bot 70:1–10

Eissenstat DM, Yanai RD (1997) The ecology of root life span. Adv Ecol Res 27:1–62

Ericsson T (1994) Nutrient dynamics and requirements of forest crops. New Zeal J For Sci 24:133–168

Fujimaki R, Tateno R, Tokuchi N (2007) Root development across a chronosequence in a japanese cedar (*Cryptomeria japonica* D. don) plantation. J For Res 12:96–102

Gower ST, McMurtrie RE, Murty D (1996) Aboveground net primary production decline with stand age: potential causes. Trends Ecol Evol 11:378–382

Graciano C, Goya JF, Frangi JL (2007) *Eucalyptus grandis* plantations in Argentina in the context of sustainable silviculture. In: Verne NC (ed) Forest Ecology Research Horizons. Nova Science Publishers, New York, pp 39–76

Guo DL, Mitchell RJ, Hendricks JJ (2004) Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. Oecologia 140:450–457

Hassiotou F, Renton M, Ludwig M, Evans JR, Veneklaas EJ (2010) Photosynthesis at an extreme end of the leaf trait spectrum: how does it relate to high leaf dry mass per area and associated structural parameters? J Exp Bot 61:3015–3028

He CX, Li JY, Zhou P, Guo M, Zheng QS (2008) Changes of leaf morphological, anatomical structure and carbon isotope ratio with the height of the wangtian tree (*Parashorea chinensis*) in Xishuangbanna, China. J Integr Plant Biol 50:168–173

Helmisaari H-S, Ostonen I, Lõhmus K, Derome J, Lindroos A-J, Meril P, Nöjd P (2009) Ectomycorrhizal root tips in relation to site and stand characteristics in Norway spruce and Scots pine stands in boreal forests. Tree Physiol 29:445–456

Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187

Hynynen J, Niemistö P, Viherä-Aarnio A, Brunner A, Hein S, Velling P (2010) Silviculture of birch (Betula pendula Roth and Betula pubescens Ehrh.) in northern Europe. Forestry 83:103–119

Imada S, Yamanaka N, Tamai S (2010) Fine-root growth, fine root mortality, and leaf morphological change of Populus alba in response to fluctuating water tables. Trees Struct Funct 24:499–506

Ingestad T, McDonald AJS (1989) Interaction between nitrogen and photon flux density in birch seedlings at steady-state nutrition. Physiol Plantarum 77:1–11

Jagodziński AM, Kałucka I (2010) Fine roots biomass and morphology in a chronosequence of young Pinus silvestris stands growing on a reclaimed lignite mine spoil heap. Dendrobiol 64:19–30

Kalliokoski T, Pennanen T, Nygren P, Sievänen R, Helmisaari H-S (2010) Belowground interspecific competition in mixed boreal forests: fine root and ectomycorrhiza characteristics along stand developmental stage and soil fertility gradients. Plant Soil 330:73–89

Konôpka B, Takáčová E (2010) Effects of liming and NPK-fertilization on the soil and fine roots in a Norway spruce stand. Ekológia 29:28–39

Kutsch WL, Wirth C, Kattge J, Noellert S, Herbst M, Kappen L (2009) Ecophysiological characteristics of large, mature trees and consequences for old-growth functioning. In: Wirth C, Gleixner G, Heimann M (eds) Old-growth forests: function, fate and value. Ecological studies, Vol 207, Part 2, Springer, Berlin, pp 57–79

Kuznetsova T, Lukjanova A, Mandre M, Lõhmus K (2010) Aboveground biomass and nutrient accumulation dynamics in young black alder, silver birch and Scots pine plantations on reclaimed oil shale mining areas in Estonia. For Ecol Manag 262:56–64

Lambers H, Poorter H (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv Ecol Res 23:187–261

Lambers H, Chapin FS III, Pons TL (2006) Plant physiological ecology. Springer, New York

Leuschner C, Harteveld M, Hertel D (2009) Consequences of increasing forest use intensity for biomass, morphology and growth of fine roots in a tropical moist forest on Sulawesi, Indonesia. Agric Ecosyst Environ 129:474–481

Liu G, Freschet GT, Pan X, Cornelissen JHC, Li Y, Dong M (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. New Phytol 188:543–553

Lõhmus E (1984) Estonian forest site types (In Estonian). Valgus, Tallinn

Lõhmus K, Oja T, Lasn R (1989) Specific root area: a soil characteristic. Plant Soil 119:245–249

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. In: Mander Ü, Wiggering H, Helming K (eds) Multifunctional land use: Meeting future demands for landscape goods and services. Springer, Berlin Heidelberg, pp 387–401

Makita N, Hirano Y, Mizoguchi T, Kominami Y, Dannoura M, Ishii H, Finer L, Kanazawa Y (2011) Very fine roots respond to soil depth: biomass allocation, morphology, and physiology in a broad-leaved temperate forest. Ecol Res 26:95–104

Marschner H (2005) Mineral nutrition of higher plants. Academic Press, London

Mediavilla S, Escudero A (2009) Ontogenetic changes in leaf phenology of two co-occurring mediterranean oaks differing in leaf life span. Ecol Res 24:1083–1090

Niklas KJ, Cobb ED (2008) Evidence for "diminishing returns" from the scaling of stem diameter and specific leaf area. Am J Bot 95:549–557

Niklas KJ, Cobb ED (2010) Ontogenetic changes in the numbers of short-vs. long-shoots account for decreasing specific leaf area in Acer rubrum (aceraceae) as trees increase in size. Am J Bot 97:27–37

Noh NJ, Son Y, Kim RH et al (2007) Biomass accumulations and the distribution of nitrogen and phosphorus within three Quercus acutissima stands in central Korea. J Plant Biol 50:461–466

Ostonen I, Lõhmus K, Lasn R (1999) The role of soil conditions in fine root ecomorphology in norway spruce (Picea abies (L.) Karst.). Plant Soil 208:283–292

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007a) Specific root length as an indicator of environmental change. Plant Biosyst 14:426–442

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

Ostonen I, Tedersoo L, Suvi T, Lõhmus K (2009) Does a fungal species drive ectomycorrhizal root traits in Alnus spp.? Can J Forest Res 39:1787–1796

Ostonen I, Helmisaari H-S, Borken W, Tedersoo L, Kukumagi M, Bahram M, Lindroos A-J, Nojd P, Uri V, Merila P, Asi E, Lohmus K (2011) Fine root foraging strategies in Norway spruce forests across a European climate gradient. Glob Chang Biol 17:3620–3632

Pearcy RW, Sims DA (1994) Photosynthetic acclimation to changing light environments: scaling from the leaf to the Ahole plant. In: Caldwell MM, Pearcy RW (eds) Exploitation of Environmental Heterogeneity by Plants: Ecophysiological Processes Above- and Belowground. Academic Press, San Diego, pp 145–174

Polglase PJ, Attiwill PM (1992) Nitrogen and phosphorus cycling in relation to stand age of Eucalyptus regnans F. Muell.: I. return from plant to soil. Plant Soil 142:156–166

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. Ecol Monogr 72:93–309

Priha O, Hallantie T, Smolander A (1999) Comparing microbial biomass, denitrification enzyme activity, and numbers of nitrifiers in the rhizospheres of Pinus sylvestris, Picea abies and Betula pendula seedlings by microscale methods. Biol Fertil Soils 30:14–19

Reich PB, Walters MB, Tjoelker MG, Vanderklein D, Buschena C (1998) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Funct Ecol 12:395–405

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339

Richter AK, Walthert L, Frossard E, Brunner I (2007) Does low soil base saturation affect fine root properties of european beech (Fagus sylvatica L.)? Plant Soil 298:69–79

Rosenvald K, Ostonen I, Truu M, Truu J, Uri V, Vares A, Lõhmus K (2011a) Fine-root rhizosphere and morphological adaptations to site conditions in interaction with tree mineral nutrition in young silver birch (Betula pendula Roth.) stands. Eur J For Res 130:1055–1066

Rosenvald K, Kuznetsaova T, Lõhmus K, Ostonen I, Truu M, Truu J, Uri V (2011b) Dynamics of rhizosphere processes and soil formation in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas. Ecol Eng 37:1027–1034

Rousseau JVD, Sylvia DM, Fox AJ (1994) Contribution of ectomy-corrhiza to the potential nutrient-absorbing surface of pine. New Phytol 128:639–644

Sellin A, Kupper P (2006) Spatial variation in sapwood area to leaf area ratio and specific leaf area within a crown of silver birch. Trees 20:311–319

Siddiqui M, Shah A, Yaqoob S (2009) Chronosequence and crown strata effects on foliar nutrient concentrations in teak (*Tectona grandis* L.f) plantations. Pak J Bot 41:3023–3034

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego

Sun Y, Gu J-C, Zhuang H-F, Wang Z-Q (2010) Effects of ectomycorrhizal colonization and nitrogen fertilization on mor-phology of root tips in a *Larix gmelinii* plantation in northeastern China. Ecol Res 25:295–302

Taylor JH, Peterson CA (2005) Ectomycorrhizal impacts on nutrient uptake pathways in woody roots. New Forest 30:203–214

Taylor AFS, Martin F, Read DJ (2000) Fungal diversity in ectomycorrhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along north-south transects in Europe. In: Schultze E-D (ed) Carbon and Nitrogen Cycling in European Forest Ecosystems. Springer, Heidelberg, pp 343–365

Tedersoo L, Kõljalg U, Hallenberg N, Larsson KH (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol 159:153–165

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M (2010a) Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. ISME J 4:465–471

Tedersoo L, May TW, Smith ME (2010b) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylo-genetic lineages. Mycorrhiza 4:217–263

Thomas SC (2010) Photosynthetic capacity peaks at intermediate size in temperate deciduous trees. Tree Physiol 30:555–573

Trocha LK, Mucha J, Eissenstat DM, Reich PB, Oleksyn J (2010) Ectomycorrhizal identity determines respiration and concentra-tions of nitrogen and non-structural carbohydrates in root tips: a test using Pinus sylvestris and Quercus robur saplings. Tree Physiol 30:648–654

Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447

Uri V, Lõhmus K, Ostonen I, Tullus H, Lastik R, Vildo M (2007) Biomass production, foliar and root characteristics and nutrient accumulation in young silver birch (*Betula pendula* Roth.) stand growing on abandoned agricultural land. Eur J For Res 126:495–506

Uri V, Varik M, Aosaar J, Kanal A, Kukumägi M, Lõhmus K (2012) Biomass production and carbon sequestration in a fertile silver birch (*Betula pendula* Roth) forest chronosequence. Forest Ecol Manag 267:117–126

Weiskittel AR, Hailemariam T, Wilson DS, Maguire DA (2008) Sources of within- and between-stand variability in specific leaf area of three ecologically distinct conifer species. Ann For Sci 65:103

White LL, Zak DR, Barnes BV (2004) Biomass accumulation and soil nitrogen availability across an 87-year-old *Populus grandiden-tata* chronosequence. For Ecol Manag 191:121–127

Withington JM, Reich PB, Oleksyn J, Eissenstat DM (2006) Comparison of structure and life span in roots and leaves among temperate trees. Ecol Monogr 76:381–397