DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
84

FINE ROOT STRUCTURE, DYNAMICS AND PROPORTION IN NET PRIMARY PRODUCTION OF NORWAY SPRUCE FOREST ECOSYSTEM IN RELATION TO SITE CONDITIONS

IVIKA OSTONEN

TARTU 2003

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
84

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
84

FINE ROOT STRUCTURE, DYNAMICS AND PROPORTION IN NET PRIMARY PRODUCTION OF NORWAY SPRUCE FOREST ECOSYSTEM IN RELATION TO SITE CONDITIONS

IVIKA OSTONEN

TARTU UNIVERSITY
PRESS

Chair of Applied Ecology, Institute of Botany and Ecology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of Doctor Philosophiae in plant ecology and ecophysiology at University of Tartu on October 10, 2003 by the Doctoral committee of Faculty of Biology and Geography of the University of Tartu

Opponent: Prof. Helja-Sisko Helmisaari, The Finnish Forest Research Institute, Finland

Commencement: Room 218, Lai 40, Tartu, on December 12, 2003, at 10.15

The publication of this dissertation is granted by the University of Tartu

Tartu Ülikooli Kirjastus
www.tyk.ut.ee
Tellimus nr. 718

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on following articles, which are referred to by their Roman numerals:

I Lõhmus K, Ivask M, Ostonen I. 1995. **Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils.** *In*: The Finnish Forest Research Institute. Research Papers 537. *Eds*. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83–87.

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

III Ivask M, Truu J, Truu M, Lõhmus K, Ostonen I. 1999. **The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia.** Baltic Forestry, 2: 32–36

IV Ivask M, Lõhmus K, Truu J, Truu M, Ostonen I. 2000. **Earthworm Lumbricidae communities in alder and aspen forest: three case studies.** Baltic Forestry 6, 1, 74–77.

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

VI Granhall U, Lõhmus K, Püttsepp Ü, Ostonen I. 2003. **Mycorrhizae in *Alnus incana*.** In: Eds. Ü. Mander and K. Lõhmus. Riparian alder forests: Their importance as buffer zones and bioenergy sources. Kluwer Academic Publishers, Dordrecht. (Accepted)

VII Ostonen I, Lõhmus K, Pajuste K. **Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce stand: comparison of soil core and ingrowth core methods.** (submitted to Ecosystems)

ABBERVIATIONS

ECM	ectomycorrhizal short roots;
NPP	net primary production (kg ha^{-1} yr^{-1});
$T_{mantle\ or\ cortex}$	thickness of mantle or cortex, measured on cross sections of roots (mm);
$D_{root\ or\ stele}$	diameter of root or stele (mm);
$PS_{mantle,\ cortex,\ or\ stele}$	the percentages of mantle, cortex and stele of the root cross section area (CSA;%);
CSA	root cross section area (mm^2);
SRL	specific root length (mm mg^{-1});
SRA	specific root area (mm^2 mg^{-1});
SEA	specific endoderm area (mm^2 mg^{-1});
RTD	root tissue density (mg mm^{-3}).

1. INTRODUCTION

Boreal forests play an important role in the global carbon budget for several reasons. First of all, boreal forests and woodlands cover ~14.5% of the earth's land surface and the soil they grow on contains a disproportionately large amount of carbon compared to other biomes (Malhi et al., 1999; Melillo et al., 1993).

Fine root dynamics plays an important role in both whole-plant carbon budgets and ecosystem-level carbon and nutrient cycling. Fine root production may account for 8% to 70% of net primary production (NPP) (Gower et al., 1995, 1996; Grier et al., 1981; Nadelhoffer and Raich, 1992; Vogt et al., 1996). Jackson et al. (1997) estimated that as much as 33% of global annual net primary productivity in terrestrial ecosystems is devoted to fine root production. Therefore, accurate estimates of fine root biomass and production and the share of fine roots in annual NPP of whole trees or stands are very important. Only a few studies report on both above- and belowground production of conifers (e.g. Ågren et al., 1980; Gower et al., 1992; 2001; Grier et al., 1981; Helmisaari et al., 2002; Keyes and Grier, 1981; Nadelhoffer et al., 1985).

In this work I have focused on fine root structure, dynamics and NPP of Norway spruce ecosystems in relation to site conditions, and some first results on grey alders are included as well. In Estonian forests the Norway spruce is the dominating tree species in 17.5% of stands, and grey alder stands account for 7.8% of the area of all stands. The average quality class for forests dominated by either species is 1.7 (Yearbook Forest 2001), which means that most of the Norway spruce and grey alder stands are growing on fertile soils. Regarding the abundance of tree species, Norway spruce occupies third and grey alder fourth place after pine and birch (Yearbook Forest 2001).

In Estonia Norway spruce usually naturally occupies the higher quality habitats, which enables higher productivity; spruce is also the climax community of our naturally developing forests. The high productivity of spruce stands is certainly one reason why spruce forests have also been studied before in Estonia, both above and below the ground (Frey, 1977; Kõlli and Kährik, 1970; Lõhmus and Oja, 1983; Palumets, 1988; 1990; 1991; 1995). Until now, the information about our forests, including spruce stands, has been lacking data on <2 mm fme-root production, and this mainly for methodological reasons. Grey alder stands have also been quite thoroughly investigated regarding production and nutrient cycling; root production has so far been evaluated indirectly (Lõhmus et al., 1996; Mander et al., 1997; Tullus et al., 1996; Uri et al., 2002). The evaluation of fine root production and turnover in grey alder stands by direct methods is in progress.

Direct measurements of fine root NPP are problematic in many ways, and the assessment methods are extremely labour-intensive. Various methods, both direct and indirect, have been used to measure fine root biomass and production —

most often by sequential coring (Ahlström et al., 1988; Fairley and Alexander, 1985; Helmisaari et al., 2002; Persson, 1978; Yin et al., 1989) or ingrowth cores (Jones et al., 2003; Makkonen and Helmisaari, 1999; Persson, 1983), minirhizotron method (Burton et al., 2000; King et al., 2002; Majdi and Nylund, 1996; Majdi and Kangas, 1997), and indirect methods such as the N budget (Aber et al., 1985; Nadelhoffer et al., 1985). A critical review of the existing root biomass and NPP assessment methods and their advantages and disadvantages was published by Vogt et al. (1998) and some aspects of problems and progress in estimating fine root production were discussed by Nadelhoffer (2000).

As fine root turnover has a strong influence on belowground allocation of C and nutrients in forest ecosystems (Arthur and Fahey, 1992; Burton et al., 2000; Hendrick and Pregitzer, 1993; Nadelhoffer et al., 1985) the methods for measuring fine root longevities have received a great attention. When using minirhizotrons, fine-root production is evaluated by dividing their average biomass (evaluated destructively) in the studied habitat by the average fine-root longevity. In effect this is the same principle that Orlov (1957) used half a century ago, but the root window methods of the time did not enable the longevity of fine roots in the ecosystem to be measured objectively. Minirhizotrons provide a nondestructive, *in situ* method for viewing and studying roots from birth to death (King et al., 2002; Majdi et al., 2001; Wells et al., 2002). In addition, tracer techniques have been used to make independent estimates of root longevity (Gaudinski et al., 2000). There are many problems with different methods; the longevity of fine roots obtained by soil and ingrowth cores and elemental budgeting is calculated, the minirhizotrons give a median lifespan, but the question of comparability of estimates obtained by different methods remains open, although the estimates remain mostly the same range. The estimates between C^{14} from the bomb carbon technique (Gaudinski et al., 2001) and the minirhizotron and soil and ingrowth core methods differ by orders of magnitude.

Processes supported by belowground carbon allocation, including fine root growth and senescence, are subject to many biotic and abiotic factors that vary spatially and temporally. It is very important to understand how different factors related to tree species and site quality affect fine root turnover and influence C allocation strategies at the tree or whole forest ecosystem scale. As a rule, the proportion of fine roots in the root system biomass increases with the decrease in the fertility of sites (Grier et al., 1981; Keyes and Grier, 1981; Olsthoorn and Tiktak, 1991; Vogt et al., 1983, 1987). The statements on the impact of soil fertility on fine root lifespan are controversial, and evidence exists for both increased (Keyes and Grier, 1981; Pregitzer et al., 1993; Vogt et al., 1986) and decreased (Aber et al., 1985; Nadelhoffer et al., 1985, 2000; Pregizer et al., 1995) fine root life spans in more fertile soils.

Concerning site quality, the morphological structure of fine roots adapts to environmental conditions (Kutschera and Lichtenegger, 2002) and the structure may be considered to be functionally optimal in these site conditions. Maximum

productivity of the stand is usually considered indicative of environmental optimality. On the level of the entire root system, optimality theory would predict that trees optimize root operation by maximizing nutrient and water uptake per plant with a minimum resource investment into the root system (Leuschner et al., 2003).

Fine root morphological characteristics have been found to vary both with soil nutrient characteristics and physical-chemical conditions (Fitter, 1985; Lõhmus et al., 1989; 1991). Changes in root morphology affect ion uptake, especially when ion mobility is low (Nye, 1973; Robinson and Rorison, 1983). Hence it is important to elucidate principles in the fine root anatomy and morphology of trees in relation to site conditions, because the link between root structure and ecological strategies of grass species is proved (Wahl and Ryser, 2000; Wilson et al., 1999).

Considering an ECM root, it consists spatially and functionally of three different parts: stele, cortex and fungal mantle, their dimensions in different habitat conditions being analysed in the current work. It is well known that the stele is responsible mainly for long-distance bi-directional transport within the plant. However, its role often remains unconsidered. At the same time, the endoderm surrounding the stele forms an almost impermeable barrier (with passage cells) separating long-distance and short-distance transport. That interface between cortex and stele can be characterized with quantitative measures. The structure and function of the two other components, (cortex and mantle) is more complicated, because they form a transition zone between plant and fungus (cortex and Hartig net), and plant and soil (mantle and/or rhizodermal cells of cortex). The Hartig net forms a highly branched network in the apoplast of the root cortex and constitutes the interface for the exchange of photoassimilates, soil water, and nutrients between the host plant and its fungal partner. It is important to reveal the share of plant and fungus in the ECM of Norway spruce, because mycorrhizal fine root turnover accounts for a considerable amount of carbon in the ecosystem carbon cycle. A few studies have examined the amount of fungal biomass in fine roots in relation to soil conditions (Ekbald et al., 1995; Hobbie and Colpaert, 2003). Retention of the integrity of the root-fungus pathway is especially important in ecosystems of this type, in which the surfaces of virtually all roots are ensheathed by a thick mantle of fungal hyphae to form ectomycorrhizas (Smith and Read, 1997)

In addition, because roots live in very close contact with all other soil biota, the relationships may be so consolidated that it is hard to distinguish between a root as a plant organ and a root as a symbiont. Rhizosphere microbial communities influence fundamental processes that contribute to nutrient cycling, plant growth, and root health. Rhizosphere communities are influenced by soil and plant factors, but little is known about the relative importance of these factors.

Carbon is translocated from aboveground biomass to the root system during root production and maintenance, and is added to the mineral soil and forest

floor carbon pools via hyphae at ectomycorrhizas and through rhizodeposition as a loss of peripheral biomass or root exudates. Microbial communities in the soil and rhizosphere and in decomposing root litter are supported by assimilates from trees (Högberg et al., 2001; Lõhmus and Ivask, 1995). The estimate of net photosynthate allocated to mycorrhizal fungi can range from 5 to 85% among different systems (Allen, 1991). Further, the extent to which the C allocated to roots is respired is also discussed in literature (Högberg et al., 2001, 2002).

This thesis summarizes for the first time the variability of fine root dimensions in different Norway spruce habitats in Estonia as well as the variability of the fine root turnover and NPP and its share in the NPP of the stand for a Norway spruce forest with high productivity. Special attention was paid to the fine-root characteristics of Norway spruce in relation to site quality, the estimates of the activity and diversity of decomposer communities were used as background information and the comparable study of grey alder fine root production is in progress.

The obtained results increase our understanding of the mechanisms through which the fine root system of Norway spruce adapts to forest site conditions, which simultaneously reflects the site productivity. The absolute and relative values could be used in models of carbon cycling in forest ecosystems.

The general objective of this thesis was to analyse the regularities of fine root structure and dynamics, and proportion in net primary production, of Norway spruce forests in relation to site conditions.

The specific objectives were:

1. to assess the NPP of fine roots and the share of fine roots in the total NPP in a high productivity spruce stand using different methods (sequential coring and ingrowth cores) (**VII**);
2. to compare two destructive root research methods (soil and ingrowth cores) regarding both the objectivity of root production estimation as well as labour intensity (**VII**);
3. to analyse the role of soil conditions on fine root anatomy (**V**), morphology (**II**) and decomposition (**I**) in Norway spruce stands;
4. to analyse the microbial activity and the structure of communities of earthworms in coniferous and deciduous forests as potential biotic factors affecting fine root variability (**III** & **IV**);
5. to analyse the variability of ECM root anatomical parameters of Norway spruce (**V**) and to compare some ECM parameters of Norway spruce and grey alder in fertile sites (**V, VI**);
6. to elucidate how root parameters on different organisation levels (cellular, tissue etc.) reflect site quality of Norway spruce forests (**II, V, VII**).

2. MATERIAL AND METHODS

2.1. Site descriptions

Norway spruce

Fine root studies were carried out on 9 permanent plots of 50 × 50 m established in 1987 in coniferous stands and located throughout Estonia according to the natural distribution of Norway spruce on automorphic soils (Table 1) Fine-root decomposition studies (**I**) were conducted in 9 stands, the study of fine root morphology (**II**) was carried out in seven of nine stands and fine root anatomy (**V**) was studied in five of nine stands. The structure and activity of the main litter decomposer communities were studied at five of the above-mentioned coniferous stands (**III**).

Each stand represents a particular forest type, site quality class, and soil type. Stand and soil characteristics are described more thoroughly in the corresponding papers.

Table 1. Characteristics of the Norway spruce sites in Estonia.

Area	Canopy composition	Forest site type	Soil type	Basal area $m^2\ ha^{-1}$	Age years	Site quality class
Roela[1, 2, 3, 4, 5]	10S +B	*Oxalis* spruce forest	Umbric Luvisol	48.9 [1979] 50.0 [2001]	40 [1979] 62 [2001]	I[a] I[b]
Voore 2[3, 5]	9S 1B	*Hepatica* spruce forest	Umbric Luvisol	33.0	50	I
Väätsa[1, 3]	9S 1B	*Aegopodium* spruce forest	Umbric Cambisol	29.7	63	I
Vigala[1, 2, 3, 5]	6S 4P	*Oxalis* spruce forest (drained)	Dystric Gleysol	35.6	43	I
Putkaste[1, 2, 3, 5]	9S 1B	*Hepatica* spruce forest (drained)	Gleyic Podzol	34.0	64	II
Pikasilla[1, 2, 3, 5]	7P 3S	*Vaccinum vitis-idea* pine forest	Sombri-Ferric Podzol	26.8	63	III
Kuusnõmme[1, 2, 3]	5S 5P	*Calamagrostis* spruce forest	Rendzic Leptosol	11.8	73	IV–V
Haanja[3]	9S 1B	*Oxalis* spruce forest	Dystric Podzoluvisol	43.2	45	I
Tipu[1, 2, 3]	8S 1P 1B	*Vaccinium myrtillus* spuce forest (drained)	Haplo-Gleyic Podzol	44.3	56	I

S – *Picea abies*, P – *Pinus sylvestris*, B – *Betula pubescens*;
1 – fine root anatomy study, 2 – fine root morphology study, 3 – decomposition study, 4 – fine root biomass and NPP study, 5 – decomposer communities in soil

Grey alder

Mycorrhizal detection of fine roots and the study of the structure and activity of the main decomposer communities were carried out in natural grey alder stands: Porijõe and Viiratsi in southern Estonia (**VI, IV**). Stand and soil characteristics are described in Table 2.

Table 2. Characteristics of the grey alder sites in Estonia.

Area	Forest site type	Soil type	Age years
Porijõe	*Aegopodium* grey alder forest, natural	Mollic-eutric Gleysol	16
Viiratsi	*Aegopodium* grey alder forest, polluted	Mollic-cumuli Gleysol	41

2.2. Soil characteristics

Ca concentration was determined photometrically and Al (with aluminum), spectrocolorimetrically from soil samples (Lõhmus and Lasn, 1990). Total N and soluble phosphorus concentration was determined by the Kjeldahl method and by lactate method, respectively. The specific surface area of the soil (S) was determined by the Puri and Murari method (1964). Methods for the determination of humus content, bulk soil density have been described in Lõhmus et al. (1989; 1995), and that for field capacity, in Brady (1990). The organic matter content in composite samples was determined in muffel oven at 360°C.

Soil decomposer communities and microbial activities (III & IV). These papers are presented here as background information about the root environment, in order to take in consideration as many components of the integrated soil-root system characteristics as possible. I was involved in collecting samples and analysing results.

The forest floor and soil samples for chemical and microbiological analysis were collected on 50 × 50 cm quadrates or on ∅ 104 mm rings. The thickness of the forest floor layer was measured. Earthworm samples were collected in May and October at the time of maximum density, greatest activity and lowest variability of individuals (Nordström and Rundgren, 1973). Earthworms were collected from soil blocks measuring 50 × 50 × 40 cm by hand, separately from the forest floor and soil layers; they were washed and identified to species. All earthworms were divided into ecological groups.

Total activity of the microbial community, as one of the essential habitat factors for earthworms, was measured using fluorescein diacetate method (Schnürer and Roswall, 1985).

2.3. Root sampling and analysis

Spruce root samples for anatomical and morphological studies were randomly collected by spading from the forest floor and from the 20 cm deep soil surface layer in October 1997 (Roela, Putkaste, Pikasilla, Kuusnõmme, Väätsa, Tipu and Vigala) and 1995 (Roela, Putkaste, Pikasilla, Kuusnõmme and Vigala). Grey alder fine roots for mycorrhizal detection were collected from Porijõe and Viiratsi stands in October 1997. Roots were washed with tap water and a small soft brush to remove mineral soil and finally rinsed with distilled water.

Anatomy study of spruce and grey alder fine roots (V, VI). Thin transverse or axial sections 5 (μm) of randomly taken spruce and grey alder short roots were cut using the freezing microtome cryostat Microm (HM 500 OM, –21°C). The embedding medium for frozen tissue specimens was TISSUE-TEC (O. C. T. 4583 compound, MILES-USA). All sections were coloured with methylene-blue and stained in "Mount-Quik-Aqueous". Root sections were examined by light microscopy (AXIOPHOT; magnification 200–800x). 110–176 (total 744) sections were measured per each spruce forest area.

Morphology study of spruce fine roots (II). Three random subsamples were taken from each sample; the number of short root tips from each set of 30 subsamples totaled 309–464. The root tips were examined under a binocular microscope, counted and photographed in order to measure their projection area and diameter. In order to ensure comparability of morphological measurements the roots were water saturated when photographed. The roots were dried at 105°C for 2 h to constant weight and then weighed. The photographs (magnification 10x) of short roots were scanned and digitized. The area of the digitized images was measured using the program PINDALA, version 1.0 (designed by I. Kalamees, Eesti Loodusfoto, Tartu, Estonia). All projected areas were calibrated separately using a standard area. To obtain the mean diameter, the diameters (*dj*) of all short root branches on photos were measured with a 1 mm step (10 mm on photos).

2.4. Root characteristics

Short root anatomical characteristics (V): thickness of the mantle (T_{mantle}) and cortex (T_{cortex}), and the diameter (D_{root}) of a transverse root section were measured in four crossing radial directions; measurements on axial sections were performed in two radial directions. The diameter of the stele (D_{stele}) and the proportions D_{stele}/D_{root} and $D_{stele}/D_{stele+cortex}$ were calculated. The percentages of the root cross-sectional area (CSA), of the mantle, cortex, and stele were calculated as follows:

$$PS_{mantle} = 100\frac{S_{mantle}}{S_{root}}, \quad (1)$$

$$PS_{cortex} = 100\frac{S_{cortex}}{S_{root}}, \quad (2)$$

$$PS_{stele} = 100\frac{S_{stele}}{S_{root}}, \quad (3)$$

where S = area of root and its compartments.

Specific endoderm area, using data (SRL) from paper **II,** was calculated as follows:

$$SEA = \pi D_{stele}\ SRL, \quad (4)$$

$$SEA = SRA(\frac{D_{stele}}{D_{root}}), \quad (5)$$

where SRA =specific root area (12) and SRL = specific root length (13).

Short root morphological characteristics (II): mean diameter, mean length and dry weight of root tip, root density and specific root area.

Geometrically the short roots of a Norway spruce can be considered as cylinders with varying diameter and length. Although the apexes of short roots are round, deviation from the shape of a cylinder can be considered insignificant, because the mean error caused by roundness of tips does not exceed 2% (Lõhmus et al., 1989). Hence, the total surface area of absorbing roots (S) is:

$$S = \pi\sum_{i=1}^{n} d_i l_i \quad (6)$$

and the volume (V) is:

$$V = 0.25\pi\sum_{i=1}^{n} d_i^2 l_i \quad (7)$$

where l = root length, d = root diameter and n = the number of short root tips.
The mean diameter (D) was calculated as:

$$D = \frac{1}{k}\sum_{j=1}^{k} d_j, \quad (8)$$

where k = the number of diameter measurements in a sample.
The mean root tip length was calculated as:

$$length = \frac{S}{n\pi D}, \quad (9)$$

where S = the surface area (1), D = mean diameter (3) and n = number of short root tips.

The mean dry weight of root tips, root tissue density (RTD), specific root area (SRA) and specific root length (SRL) were calculated as follows:

$$weight = \frac{M}{n}, \quad (10)$$

$$RTD = \frac{M}{V}, \quad (11)$$

$$SRA = \frac{S}{M}, \quad (12)$$

$$SRL = \frac{L}{M}, \quad (13)$$

where M = the dry weight of the sample, n = number of root tips, V = the volume of short roots in the sample (7) and S = the surface area (6).

Fine root biomass, NPP and decomposition (I, VII). Sequential soil coring and ingrowth core methods were used to estimate fine root (<2 mm) biomass and NPP. Fine root samples were taken during four consecutive growing seasons in 1996–1999.

Sequential cores. Twenty soil cores (volumetric samples, core diameter 38 mm) per sampling were taken monthly during the period June-1996 to June-1997 (140 in total). The soil cores were divided into seven layers by depth: forest floor, 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–30 cm, 30–40 cm.

Ingrowth cores. In the root ingrowth core method, a total of 105 ingrowth core samples were collected during the growing seasons of 1997–1999, once after one year and three times in the second and third years. In December 1996, 192 mesh bags (∅ 40 mm, mesh size 6 mm) were installed in the study area in a regular pattern in two groups of parallel transects. 16 ingrowth cores were installed per transect; the distance between the ingrowth cores and between the transects was 1 m. Ingrowth cores were inserted into the soil to a depth of 30 cm from the surface of the forest floor. A 1-cm-thick forest floor layer was put on top of each core. Mesh bags were filled with root-free soil according to the soil's genetical horizons. Sampling was carried out in November 1997, June, August and November 1998 and June, September and December 1999. As in the soil core method, ingrowth cores were divided into depth layers, excluding the 30–40 cm layer.

Sample processing. All obtained subsamples were transported to the laboratory and stored frozen (–18°C) until analysis. In the laboratory, spruce roots from samples taken by both methods were washed free of soil and separated into living and dead roots. Both living and dead roots were separated into two diameter classes: d<1 mm and 1 mm ≤ d <2 mm (further 1–2 mm). The dry mass was determined after drying of fine root samples at 70°C to constant mass. Ash content was determined from composed fine root samples of month.

Fine root NPP calculation. The total fine root production was calculated by balancing the living and dead root biomass compartments according to the decision matrix presented by Fairley and Alexander (1985):

		LIVE		
		increase	**decrease**	
			$\Delta B_{dead} > \Delta B_{live}$	$\Delta B_{live} > \Delta B_{dead}$
DEAD	**increase**	$P=\Delta B_{live}+\Delta B_{dead}$	$P=\Delta B_{live}+\Delta B_{dead}$	P=0
	decrease	$P=\Delta B_{live}$	P=0	

Root turnover rate (yr^{-1}) was calculated as annual root production divided by mean fine root biomass. Mean, minimum or maximum root biomass is used to calculate fine root turnover rate (Eissenstat and Yanai, 2002). We used the mean fine root biomass instead of minimum or maximum biomass to avoid large fluctuations during the vegetation period. Fine root longevity was calculated as the reciprocal of root turnover rate (yr^{-1}).

Fine root proportion in total NPP was calculated using fine root biomass data (Table 1 in paper **VII**) determined twenty years ago at the same study area and the fine root turnover rate calculated in this study.

Fine root decomposition. For Norway spruce, the initial material for decomposition was collected at high quality spruce stands: finest roots (<1 mm in diameter) at the Roela site and fine roots (<2 mm) at the Haanja site; for Scots pine, the fine roots were collected at the low quality pine stand at Nõva. The litterbag method was used. Each bag contained 1000 mg of finest or about 500 mg of fine roots. One hundred bags of finest roots were incubated randomly under the forest floor and in the subsequent 10 cm soil layers down to a depth of 40 cm in the Roela site in July 1986. The litterbags of fine roots were incubated in soil at a depth of 10 cm in July 1989. The bags were collected once or twice a year except for Roela (Voore1) and Voore 2 sites, where the seasonal dynamics were investigated. In all initial and decomposing samples, oven-dry weight, ash and energy content, and nitrogen concentration were determined

2.5. Statistical analysis and data processing

The normality of variables was checked by Lilliefors and Shapiro-Wilk's tests. Except for the PS_{cortex} and SEA, the root parameters were not normally distributed. To normalize the variables, root diameter and T_{cortex} were log transformed, T_{mantle}, PS_{mantle}, PS_{stele} and D_{stele} were repeatedly square-root, log or arcsine transformed. The live/(live + dead) proportions for different root diameter classes were normalized by arcsin-transformation. However, group variances of PS_{mantle} and PS_{stele} were inhomogeneous and the group means were compared by 95%-confidence intervals. For the rest of the parameters of paper **V**, fine root live/(live + dead) proportions in paper **VII**, and root functional parameters in paper **II**, multiple comparison of means was applied using Tukey test for unequal n.

Nonparametric Gamma correlation coefficients were calculated to estimate strength and significance of relationships, as one variable (site quality class) was in an ordinal scale and the data contained many tied observations. Linear regression analysis was performed to estimate the relationship between fine root biomass and soil depth.

Principal component analysis (PCA) and redundancy analysis (RDA) (CANOCO programme: Ter Braak, 1987) were used to detect relationships between root characteristics and soil parameters. The significance of RDA analysis results was tested with a permutation test ($p<0.01$). Specific root length was not included in the RDA analysis, but values were compared to literature data. In all cases, level of significance $\alpha = 0.05$ was accepted.

3. RESULTS

3.1. Fine root ectomycorrhizas of Norway spruce and grey alder in different soils (V, VI)

3.1.1. Proportions of mantle, cortex and stele of Norway spruce ectomycorrhizas (V)

All analyzed short root tips of Norway spruce in different stands were colonized by ECM fungi; the mantle and the Hartig net were found in all cases. The colour of the mantle varied from white to black. Several quantitative characteristics were measured in fine roots at Roela, Putkaste, Pikasilla, Vigala, and Kuusnõmme: thickness of the mantle (T_{mantle}) and cortex (T_{cortex}) and diameter of the root (D_{root}), the means varied from 16.5 ± 0.6 to 29 ± 1.3 µm, 83.9 ± 1.7 to 108.4 ± 2.4 µm, and 315.7 ± 5.7 to 422.5 ± 6.5 µm, respectively (Table 2 in paper V). The number of cell rows in the cortex (4-6) did not vary between different spruce stands. Significant differences between stands were found and are indicated by different letters in Figure 1.

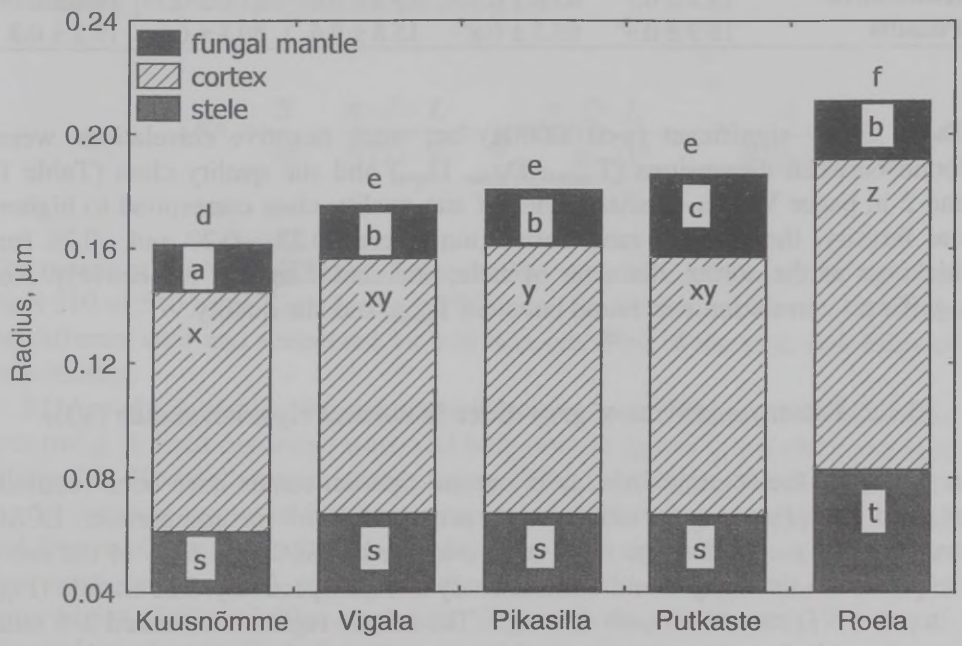

Fig 1. Mean characteristics of Norway spruce short roots in five study areas. Letters: a, b, c denote significant differences between mean thickness of fungal mantle; x, y, z, between mean thickness of cortex; s, t, between mean of stele radius, and d, e, f, between means of root radius.

Mean proportions of fungal mantle (PS_{mantle}), cortex (PS_{cortex}), and stele (PS_{stele}) of the spruce root CSA varied from 17.7 to 28.1%, from 58.9 to 66.9%, and from 13.4 to 15.8%, respectively (Table 3).

The mean proportion of cortex and stele in the CSA of cortex+stele (mantle excluded), $S_{cortex}/S_{cortex+stele}$ and $S_{stele}/S_{stele+cortex}$ varied from 81 to 82% and from 18 to 19%, respectively (Table 3); no statistically significant differences between stands were found for either characteristic.

Table 3. Mean proportions (%) of fungal mantle, cortex and stele of short root cross-sectional area and mean proportions (%) of plant tissues of Norway spruce short roots (fungal mantle excluded). Significant differences (Tukey test, $p<0.05$) are indicated by different letters.

Area	Mantle included			Mantle exluded	
	PS_{mantle} %	PS_{cortex} %	PS_{stele} %	Cortex %	Stele %
Roela	17.7 ± 1.0^{a}	66.9 ± 1.0^{x}	14.9 ± 0.7	81.6 ± 0.8	18.4 ± 0.8
Putkaste	28.1 ± 1.0^{b}	58.9 ± 0.9^{y}	13.4 ± 0.7	81.6 ± 0.8	18.4 ± 0.8
Vigala	21.3 ± 0.8^{a}	64.9 ± 0.8^{x}	14.9 ± 0.6	81.7 ± 0.8	18.3 ± 0.7
Kuusnõmme	19.5 ± 0.7^{a}	65.8 ± 0.7^{x}	15.2 ± 0.6	81.6 ± 0.7	18.4 ± 0.6
Pikasilla	19.9 ± 0.9^{a}	65.2 ± 0.8^{x}	15.8 ± 0.6	80.8 ± 0.7	19.2 ± 0.7

There highly significant ($p<0.000001$) but weak negative correlations were found between dimensions (T_{cortex}, D_{stele}, D_{root}) and site quality class (Table 1 and 2 in paper V). As smaller values of site quality class correspond to higher site fertility, the Gamma rank correlations were –0.22, –0.20 and –0.26 for thickness of the cortex, diameter of stele, and root diameter, respectively. No significant correlation was found between T_{mantle} and site quality.

3.1.2. Ectomycorrhizas of grey alder in natural riparian stands (VI).

In this work the ectomycorrhizas in natural riparian stands have been focused on, and the planted grey alder stands are considered for comparison. ECM structures of grey alder fine roots were observed in 86% and 67% of the sub-samples from the Porijõe and Viiratsi study-sites, respectively. The mantle (Fig 1 in paper **VI**) may be smooth or rough. The mantle region is extended 2–8 mm from the root apex in mature grey alder trees. The surface of the mantle has a net-like structure of branched and lobed, densly adjoining hyphae. Loose hyphae extending from the mantle occurred abundantly, forming an extra-matrical mycelium. In some (Estonian) samples, rhizomorphs, i.e. multihyphal linear aggregates, were seen. The figure refers to the extent of ECM coloniza-

tion across the whole fraction of the fine roots studied and not only the root tip, the most extensively colonised region. Grey alder ECM were predominantly light-brown, purple-brown and deep-brown in natural sites, the abandoned field being dominated by whitish ones. Aggregates of ramified ectomycorrhizal root-tips (Fig 2 in paper **VI**), 20–50 mm in diameter, occurred in both the Porijõgi and Viiratsi sites.

3.2. The role of soil conditions in fine root morphology of Norway spruce (II)

The results of principal component analysis for root characteristics (RTD, SRA, D, mean root tip length and mean root tip dry weight) show that two axes account for 72% of the total variation of morphological parameters of short ectomycorrhizal roots, while three axes account for 99%. RTD and SRA were correlated with the first axis, mean root tip length and dry weight with the second axis, and the mean diameter with the third axis. The soil variables explained the largest proportion of the variation associated with fine root morphological state (RTD and SRA). SRA - a characteristic of root-soil contact, is inversely proportional to diameter and RTD.

$$SRA = \frac{S}{M} = \frac{\pi \cdot D \cdot L}{RTD \cdot V} = \frac{\pi \cdot D \cdot L}{RTD \cdot \frac{\pi \cdot D^2}{4} \cdot L} = \frac{4}{RTD \cdot D} \qquad (14)$$

The means of SRA and RTD of different sites varied from 29 to 42 $m^2\ kg^{-1}$ and from 310 to 540 kg m^{-3}, respectively. Diameter varied from 0.26 to 0.32 mm in the different sites and accounted for less than one third of the total variability of root density.

RDA analysis of 11 soil parameters: humus content (%), specific soil surface area ($m^2\ g^{-1}$), field capacity (mm), soil bulk density (g cm^{-3}), pH (KCl and H_2O dilution's), N and Ca concentrations (mg 100 g^{-1}), Ca/Al and C/N ratios, and the decomposition rate of fine roots (expressed as% of initial weight, d<2 mm), and five root characteristics are displayed on a biplot (Fig 2) which presents their relationships. RDA results of soil variables and sites are displayed on the same biplot. Soil variables explained 28% and sites 29% of the total eco-morphological variability of short roots ($p<0.01$). Soil humus content, a general measure of soil fertility, described most of the total variability (10%), followed by the soil C/N ratio, field capacity, pH_{H2O}, specific soil surface area and pH_{KCl}. Other soil characteristics did not account for additional variation. Field capacity and specific soil surface area (an indirect measure of the water regime) reflected

soil-water conditions best; without them the total variance explained by all soil characteristics decreased by one fifth (5.3%). Hence, soil nutrient regime, followed by water regime, described by the humus content, field capacity and specific soil surface area, are the most important soil variables in terms of explaining the variability of characteristics of short roots in Norway spruce.

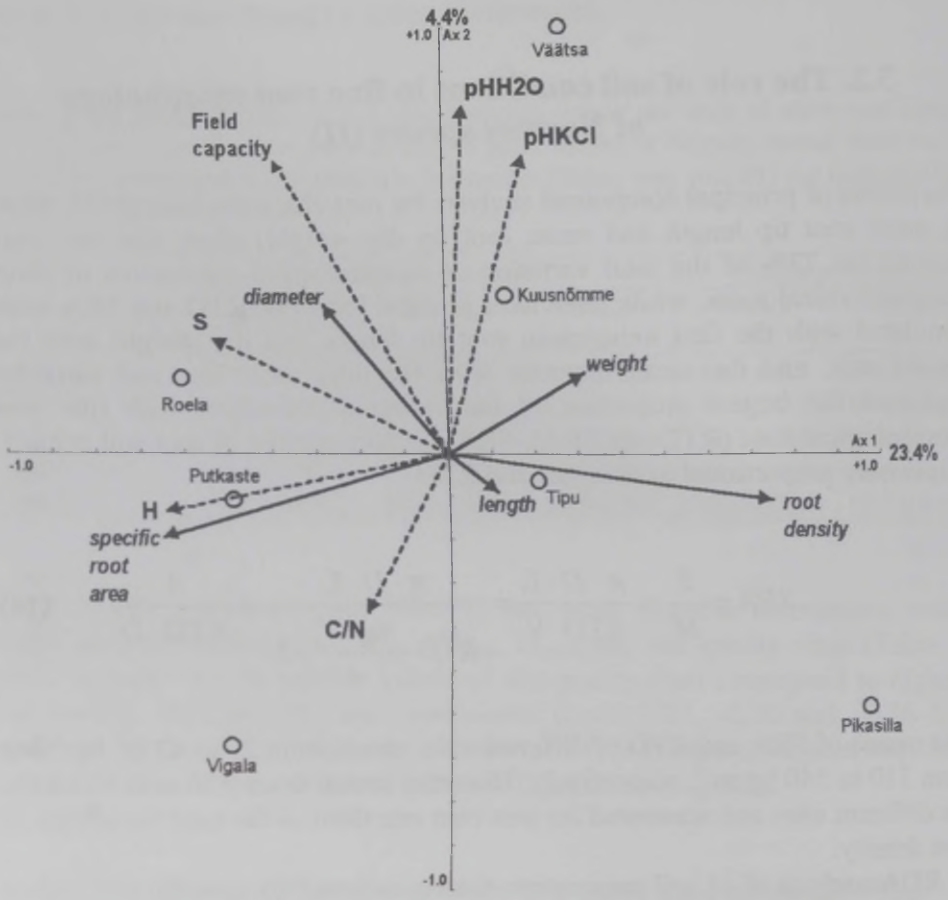

Fig 2. Biplot of RDA analysis. Solid lines are root characteristics, dashed lines are soil parameters and circles are different study areas and corresponding forest sites.

Three significantly different groups were formed by multiple comparison of means with respect to RTD: 1) Roela, Putkaste, Vigala; 2) Tipu, Väätsa, Kuusnõmme and 3) Pikasilla, and two groups with respect to specific root area: 1) Roela, Putkaste, Vigala and 2) Tipu, Väätsa, Kuusnõmme, Pikasilla (Table 4). The RTD of the first group of sites, indicated by a superscript, was significantly smaller, and specific root area correspondingly higher, than the other stands.

Table 4. Mean RTD (kg m^{-3})and SRA (m^2 kg^{-1}) of Norway spruce fine roots in seven spruce stands. Letters denote significant differences between means.

Area	RTD kg m^{-3}	SRA m^2 kg^{-1}
Roela	310 ± 10^a	40.9 ± 1.1^x
Putkaste	320 ± 10^a	42.0 ± 1.1^x
Vigala	340 ± 20^a	42.4 ± 0.9^x
Tipu	410 ± 10^b	34.8 ± 1.1^y
Väätsa	410 ± 20^b	31.5 ± 1.1^y
Kuusnõmme	400 ± 10^b	33.6 ± 1.2^y
Pikasilla	540 ± 20^c	29.4 ± 1.0^y

These stands are associated with optimal soil conditions, connected with the first ordination axis (Fig 2). Root density decreases and specific root area increases from right to left along the first axis.

Analogously to SRA, which characterizes root absorbing area for water and mineral nutrients, the specific endoderm area (SEA) can be calculated; this characterizes the surface area of the stele, where water and nutrients enter the transport system of the plant. The root tissue density is assumed to be homogenous in ECM volumes. The mean weight and length of root tips (SRL) values of paper **II** and mean diameter of stele in different sites of paper V were used to calculate SEA as follows:

$$SEA = \pi D_{stele}\, SRL \tag{15}$$

$$SEA = \left(\frac{D_{stele}}{D_{root}}\right) SRA \tag{16}$$

SEA varied from 13.7 to 20.8 mm^2 mg^{-1} and statistically significant differences between study areas were found (Table 5).

Table 5. The mean specific endoderm area (SEA) of short roots of Norway spruce. Letters denote significant differences between stands.

Area	Site quality class	SEA mm^2 mg^{-1}
Roela	I^a	20.8^a
Putkaste	II	18.3^a
Vigala	I	20.1^a
Kuusnõmme	IV–V	13.7^b
Pikasilla	III	15.7^b

3.3. Fine root biomass, production and turnover in a fertile Norway spruce stand: comparison of soil core and ingrowth core methods (VII)

3.3.1. Fine root bio- and necromass estimation by sequential coring and ingrowth cores.

The vertical distribution of Norway spruce fine roots. In the case of both methods, sequential cores and 3rd year of ingrowth cores, the majority (about 90%) of living fine roots were found to be located in the forest floor, and top 20 cm mineral soil horizon (Fig 3). The impact of sampling time on relative vertical distribution of fine root biomass was insignificant ($p>0.05$) in all cases.

In the 3rd year after establishment, the fine root biomass in ingrowth cores had not reached the level inherent to the stand, but proportionally the roots were distributed similarly along the depth gradient.

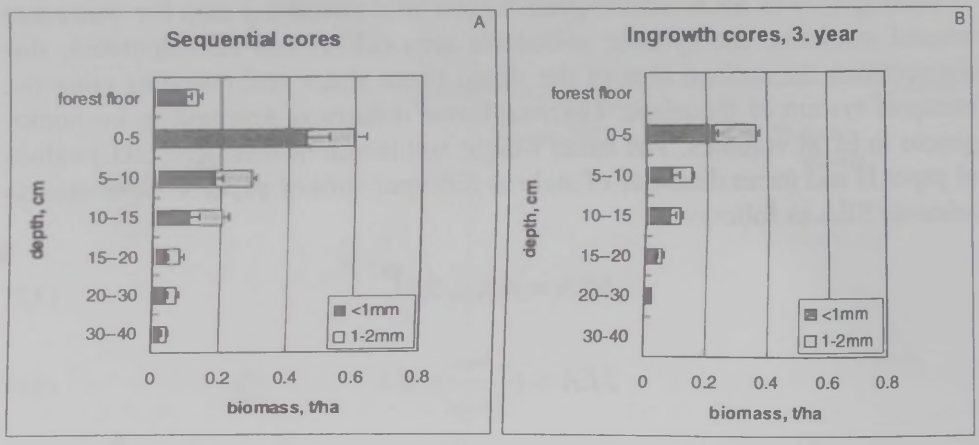

Fig 3. The vertical distribution of Norway spruce fine roots of different diameter classes in sequential cores and in ingrowth cores of up to 40 cm depth of mineral soil. Bars indicate standard errors.

Bio- and necromass of different fine root diameter classes. The mean total biomass in soil cores and third year ingrowth cores was 1420 ± 170 kg ha^{-1} and 700 ± 105 kg ha^{-1}, respectively. The mean biomass in 3rd year ingrowth cores was two times smaller than in soil cores (Table 3 in paper **VII**). The mean total necromass in soil cores and 3rd year ingrowth cores was 1540 ± 120 kg ha^{-1} and 685 ± 50, respectively.

In soil cores the share of <1 mm living roots formed 2/3 of the <2 mm root biomass; that is similar to the ratio in 3rd year ingrowth cores (62%) (Fig 4;

Table 3 in paper **VII**). The share of <1 mm dead roots was greater than that of living roots, and their ratio was similar in soil cores and 3rd year ingrowth cores (Fig 4; Table 3 in paper **VII**).

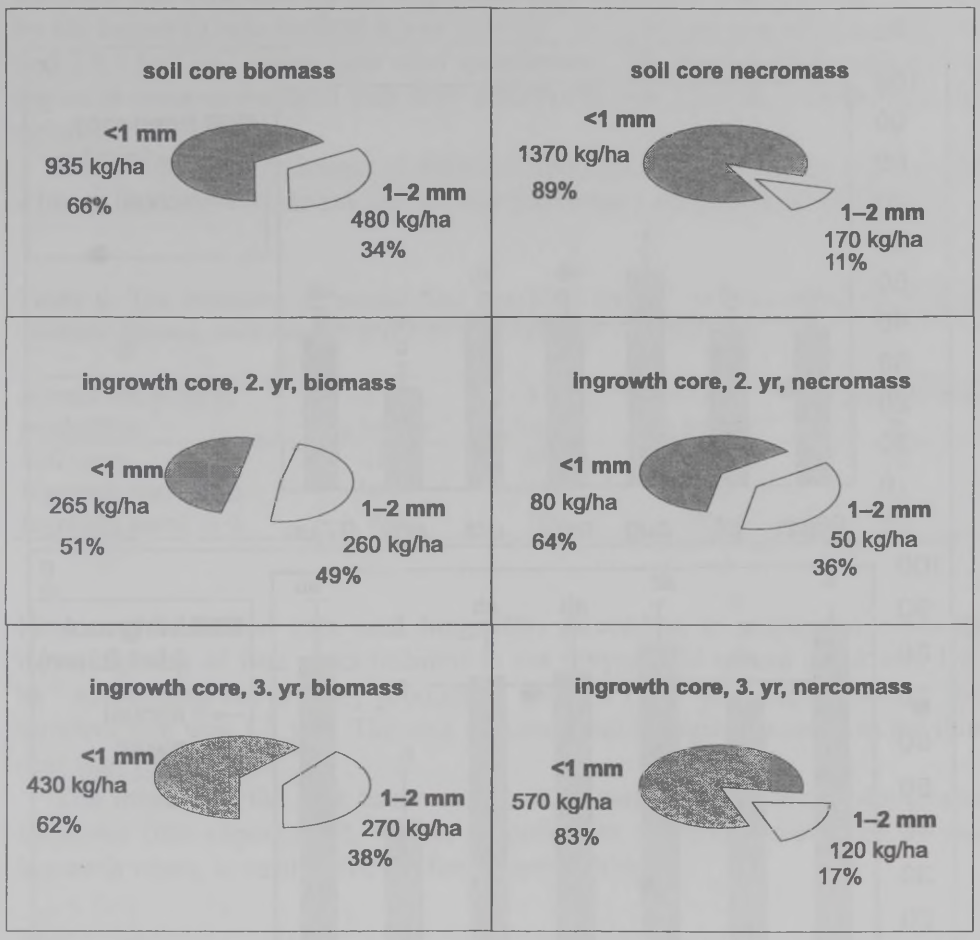

Fig 4. The proportions in different Norway spruce fine-root diameter classes (%) of bio- and necromasses, estimated by soil cores and ingrowth cores (2nd and 3rd year).

Seasonal variability of living part of fine roots. The biomass of finest (<1 mm) roots varied from 440 (Nov.) to 1315 (Okt.) kg ha^{-1} and the biomass of roots of diameter 1–2 mm varied from 190 (Nov) to 675 (July and Oct.) kg ha^{-1}.

The mean share of living fine roots in the total (live + dead) fine root mass varied seasonally for both <1 mm and 1–2 mm diameter classes (Fig 5A and 5B). The living part was higher in August for both <1 mm and 1–2 mm diameter fractions, and for 1–2 mm diameter fraction also in June, in both 1996

and 1997. The proportion of living roots in total root mass decreased significantly in November. Thus, the best time to collect fine root samples for biomass data is the autumn: September – October (Fig 5A and 5B).

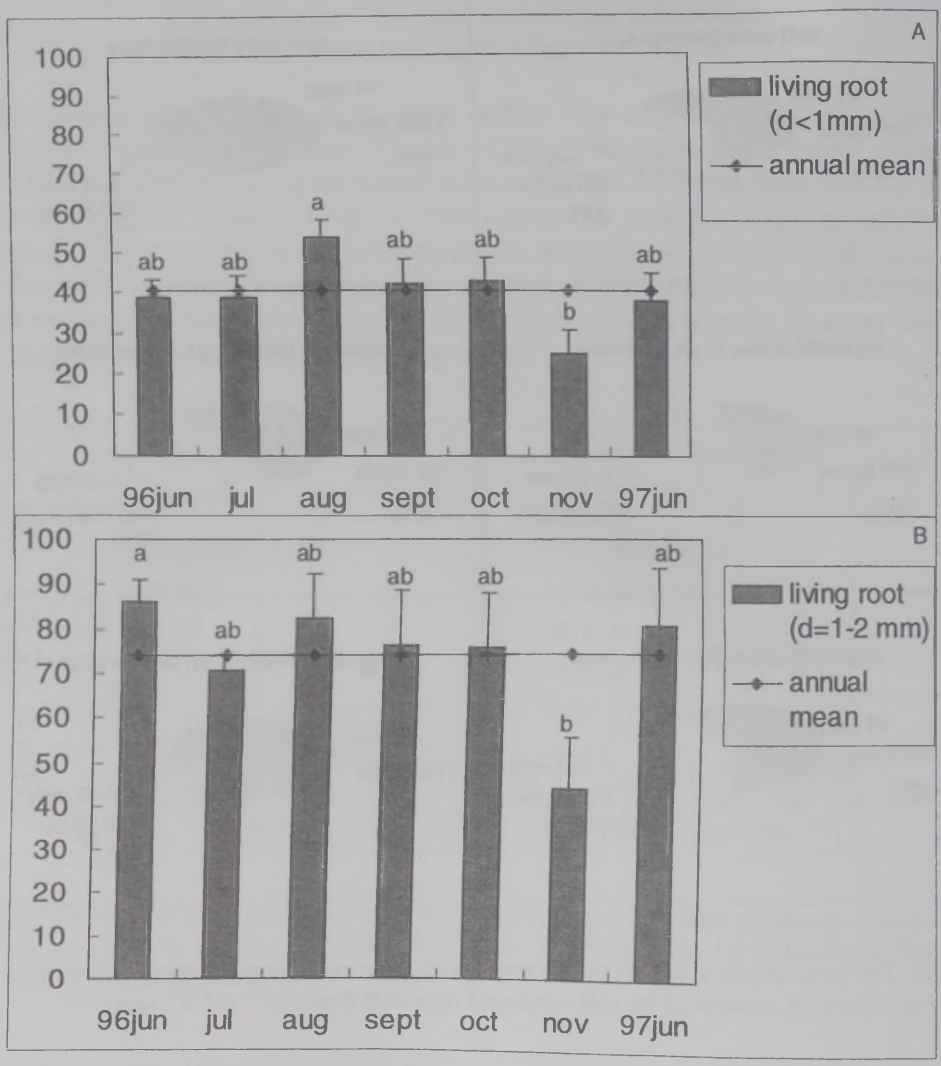

Fig 5. The seasonal variation in proportions of living fine root mass (d<1 mm, d=1–2 mm) within the total diameter class and the annual mean. Letters denote significant differences between the means of different months (Tukey test for unequal N, $p<0.05$). Bars indicate standard errors. The live/(live+dead) proportions for the 1–2 mm roots were normalised by arcsin-transformation. In Fig 5B the means and standard errors for untransformed data are presented; for the multiple comparison of means (Tukey test for unequal N, $p<0.05$), the normalised data is used.

3.3.2. Fine root NPP and its share in total NPP

Fine root NPP. The fine root (<2 mm) production estimated by the two methods was different: by the sequential core method it was 2.5 t ha^{-1} yr^{-1} and by the ingrowth core method it was 0.9 t ha^{-1} yr^{-1} (second year after installation) and 1.0 t ha^{-1} yr^{-1} (third year after installation). The annual NPP estimated by ingrowth cores in the third year after installation was 2.5 times smaller than that estimated by soil cores.

The annual NPP estimates of different fine root diameter classes (<2 mm and <1 mm; 1–2 mm was calculated as their difference) are presented in Table 6.

Table 6. The estimates of annual fine root NPP kg ha^{-1} yr^{-1} according to different diameter classes, estimated by soil core and ingrowth core methods.

Annual net primary production	<1 mm, kg ha^{-1} yr^{-1}	1–2 mm, kg ha^{-1} yr^{-1}	<2 mm, kg ha^{-1} yr^{-1}	<1 mm/ <2 mm, %
Soil cores	1830	680	2510	73
Ingrowth cores, 2. a.	450	440	890	51
Ingrowth cores, 3. a.	865	100	965	90

Fine root turnover rate and longevity. According to sequential cores the mean biomass of fine roots (<2mm) in the 60-year-old spruce stand was 1.4 t ha^{-1} and annual net primary production was 2.5 t ha^{-1} yr^{-1}; the calculated root turnover rate was 1.8 yr^{-1}. The root turnover rate in ingrowth cores in the third year was 1.4 yr^{-1}.

The inverse of the root turnover rate is, in turn, a measure of average root longevity (life expectancy), which, according to sequential cores and 3rd year ingrowth cores, is smaller for the finest roots (Table 7).

Table 7. The mean calculated turnover rate and longevity (yr) of fine roots.

Method	Turnover rate (yr^{-1})		Longevity, yr	
	<2 mm	<1 mm	<2 mm	<1 mm
Soil cores	1.8	1.9	0,57	0,51
Ingrowth cores, 2. a.	1.7	1.7	0,59	0,59
Ingrowth cores, 3. a.	1.4	2.0	0,73	0,50

Combined method. In order to reduce the volume of work a combined method for the estimation of fine root production was developed. Since the relative measures in ingrowth cores (Fig 4 and Tables 6, 7.) stabilised by the third year, the fine root production was calculated by multiplying the turnover rate of

ingrowth cores by the fine root biomass estimated by soil coring (Fig 6). For the fine roots (d<2 mm), the biomass was on the same level from June to October, with the values in June smaller, but statistically insignificantly so. The fine root biomass is significantly lower in November. The best time to collect fine root samples for biomass data is the autumn (Fig 5AB and 6).

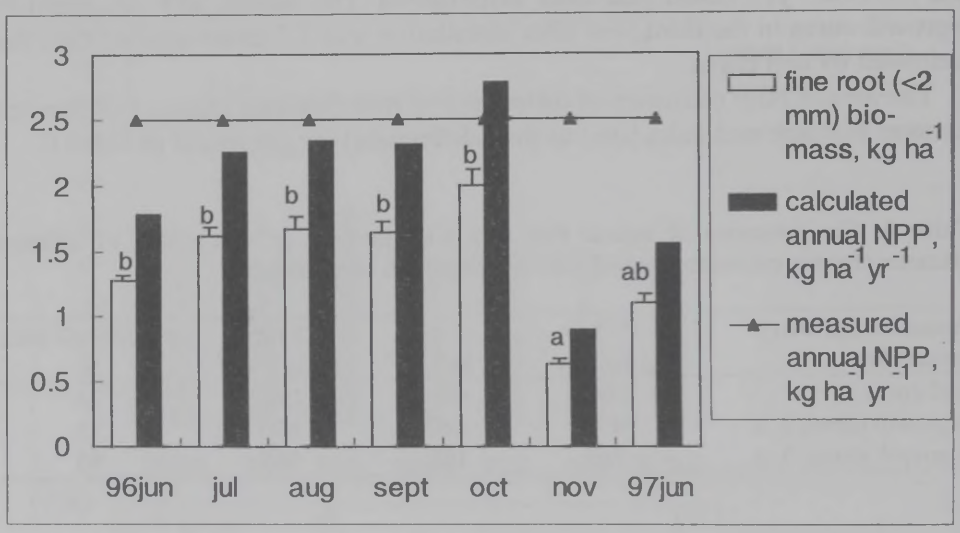

Fig 6. The dynamics of fine root biomass (<2 mm) estimated by sequential coring, and fine root annual NPP calculated by the combined method (turnover rate of 3rd year ingrowth core (yr^{-1}) × fine root biomass measured by sequential cores (t ha^{-1}) = annual NPP (t ha^{-1} yr^{-1})). The line denotes fine root annual NPP measured by sequential coring. Letters indicate significant differences between the mean fine root biomasses of different months; bars indicate standard errors.

The suggested combined method gave an 11% higher estimate of fine-root-measured annual NPP in our study stand for fine root biomass in October, and an 8% smaller estimate for fine root biomass in September (Fig 6). According to Fig 6., for the combined method is suitable for assessing fine root biomass in the vegetative period in any month, nevertheless, in the given climatic conditions, biomass is smaller in June. At that time the detrimental effects of winter can not be ruled out and the intensive allocation of assimilates to growing shoots is in progress.

The share of fine roots in total NPP. In the investigated spruce stand, at the age of 40 years the biomass of fine roots (d<2mm) was 1.64 t ha^{-1}. Using the turnover rate of fine roots found in the 60-year-old stand to estimate the fine root production in the 40-year-old stand, the estimate is 2.8 t ha^{-1} yr^{-1} (Fig 7). Hence, in the highly productive spruce stand the annual net primary production

of trees at the age of 40 years is estimated as 21.4 t ha^{-1} yr^{-1}, the share of the below-ground part forming 31%. Fine roots accounted for around 13% of the net primary production (Fig 7).

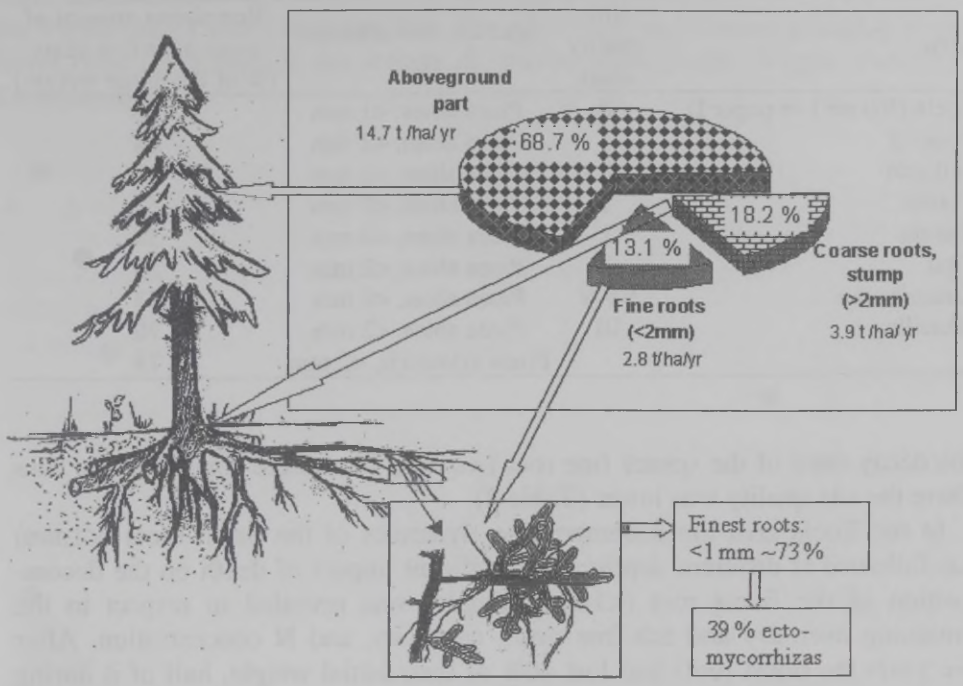

Fig 7. The annual NPP (t ha^{-1} yr^{-1}) and its proportions (%) in a middle-aged *Oxalis*-Norway spruce stand.

3.4. Fine root decomposition and decomposer communities (I, III, IV & VII)

Fine root decomposition depends on substrate quality, decomposer communities and environmental conditions.

The initially similar fine root (<2 mm) material at eight study sites had lost from 27% to 51% of its dry weight after five years (Table 8).

Table 8. Amount of fine roots remaining (%) after five years decomposition. In Roela the samples of finest roots were located 10, 20, 30 and 40 cm below the forest floor; in the other seven study areas, at a depth of 10 cm.

Area	Site quality class	Species, root diameter class	Remaining amount of roots after five years (% of the initial weight)
Roela (Voore 1 in paper I)	I[a]	Picea abies, <1 mm	60
Voore 2	I	Picea abies, <2 mm	54
Putkaste	II	Picea abies, <2 mm	49
Vigala	I	Picea abies, <2 mm	61
Haanja	I	Picea abies, <2 mm	54
Tipu	I	Picea abies, <2 mm	53
Kuusnõmme	VI–V	Picea abies, <2 mm	73
Pikasilla	III	Picea abies, <2 mm	70
		Pinus sylvestris, <2 mm	73

The decay rates of the spruce fine root (<2 mm) litter were smaller at the sites where the site quality was lower (Table 8).

In the Roela area the decomposition dynamics of the finest roots (<1mm) was followed at different depths; no significant impact of depth on the decomposition of the finest root (<1mm) samples was revealed in respect to the remaining oven-dry and ash-free mass, caloricity, and N concentration. After five years the finest roots had lost 40% of their initial weight, half of it during the first year (Table 1A in paper **I**).

In Pikasilla study area, fine root litter decomposition of spruce and pine were compared; the decay rates were similar despite differences in the initial N, ash and lignin contents; this means that decomposition rate depends mainly on decomposer communities.

The earthworm community is the most important component of the soil fauna attending in the regulation of decomposition and nutrient cycling. All earthworm species are participating in the decomposition process and in the mixing of organic and inorganic components (Edwards, 1985). In the Porijõgi study area, as a non-contaminated grey alder stand, the number of individuals (per m^2) and species (6–7) was higher than in the heavily pig slurry polluted grey alder stand at Viiratsi. The structure of the earthworm community differed significantly between these two alder stands: the grassland species were not found in the contaminated area (they are not able to live in soil contaminated by slurry) and the typical inhabitant of decaying manure and compost, *Eisenia foetida*, was present.

In spruce forests the numbers of earthworm individuals and species were highest in Roela (named Voore in paper **III**) study area. In the Kuusnõmme study area the forest floor layer on the surface of the soil is missing, but the ground vegetation of grasses is abundant. In this stand the numbers of earth-

worm individuals and endogeic species were the highest, and typical grassland species dominated. The forest floor layer was thickest in Vigala (Table 2 in paper **III**), but the number of earthworms in the forest floor was low. On the other hand, the activity of microbes was highest in the forest floor sample from the Vigala site (Table 3 in paper **III**). The activity of microbes was higher in the forest floor layer than in the soil at all studied sites (Roela, Vigala, Putkaste, Kuusnõmme).

4. DISCUSSION

4.1. Norway spruce fine root parameters

In the course of investigations of ectomycorrhizas and fine-roots in spruce stands growing in many different site types, a large amount of data, both direct measurements and derived values as well as subjective impressions, have been collected. To understand the meaning of fine root parameters, to follow their dynamics or stability, I tried to systematise them as direct measurements, derived and relative root characteristics.

Parameters based on direct measurements. The mycorrhizal structures of ECM roots of Norway spruce have been more thoroughly analysed from the mycological side: morphotyping of ectomycorrhizal roots by macro- and microscopical features (Agerer, 1987–97) and the rapid development of molecular techniques has allowed examination of ECM communities below-ground or even in one ECM root tip (Horton and Bruns, 2001). Data on structural parameters of the plant components of ECM roots and their impact on physiological processes is still scarce. Direct measurements of ECM carried out in the present work are mainly related to size.

The effects of the fungal symbiont on short-root anatomical-morphological structure appeared in all cases, because all the measured 3500 short root tips in anatomical and morphological studies in seven areas throughout Estonia were ectomycorrhizal. The range of the ectomycorrhizal parameters gave us the characteristic dimensions for spruce ECM roots in Norway spruce stands on automorphic soils within their natural distribution area in Estonia (Table 9).

Table 9. The total variation of measured ECM characteristics in Norway spruce stands in Estonia.

Area	The number of cell rows in cortex	Thickness of mantle, µm	Thickness of cortex, µm	D_{stele}, µm	D_{root}, µm	Weight of root tip, µg	Length of root tip, mm
Roela	4–6	0–60	21–213	94–154	252–638	163–850	0.92–2.91
Putkaste	4–6	0–70	55–189	94–139	180–594	185–846	0.93–3.58
Vigala	4–6	0–43	10–180	68–139	175–600	153–569	0.79–2.95
Väätsa	–	–	–	–	–	215–967	0.68–2.88
Tipu	–	–	–	–	–	213–923	0.97–2.87
Pikasilla	4–6	0–60	45–188	75–135	195–612	267–664	1.04–3.37
Kuusnõmme	4–6	0–38	46–163	83–146	143–565	208–627	0.83–2.37

The number of cell rows (4–6) in the cortex did not vary between stands (Table 9), hence it could be considered to be inherent to Norway spruce. Thus, the

thickness of the cortex depended mainly on cell size, which is in general concordance with the results of Eissenstat and Achor (1999), who found that larger root diameter among first-order roots of citrus rootstocks was caused by larger, rather than by more numerous cells in the cortex.

Concerning the dimensions of the stele, cortex and fungal mantle, the last had the highest variability (**V**), which is most probably due to the high diversity of fungal symbionts. The number of different species and morphotypes of ectomycorrhizal fungi found in the roots of Norway spruce is quite high (Egli et al., 1993; Söderström and Bååth, 1978), but the general rule is that a few common ECM species account for most (>50%) of the mycorrhizal abundance and are widely distributed, whereas the majority of species are only rarely encountered (Erland and Taylor, 2002; Grogan et al., 2000; Jonsson et al., 1999; Peter et al., 2001).

The fungal sheath can vary in radial thickness from 10 to 100 μm (Rousseau and Reid, 1989) and Harley and Smith (1983) reported the average size of the fungal sheath of ectomycorrhizas in temperate forests to be 20–40 μm.

The cortex is the main zone of exchange of mineral and organic nutrients between host and fungal symbiont. Bücking and Heyser (2001) noticed that cortical cell nuclei showed a high carbohydrate sink capacity, indicating increased metabolic activity in cortical cells. A larger cortex might provide a larger store for minerals, proteins or lipids (Luxova, 1992). The significant positive correlation between site quality class, on one hand, and thickness of the cortex, as well as the diameter of the stele (**V**) and root (**II** and **V**), on the other, indicate the positive impact of site fertility on the dimensions of root tissues of spruce ectomycorrhizas.

The sensitivity of stele radius to stand differences was smaller than for other investigated anatomical variables. Also, Enstone et al. (2001) noticed, in the case of *Pinus taeda,* that the stele dimensions remained approximately constant. In our study, D_{stele} was larger only in the highly productive Roela spruce stand, where the mean D_{root} was the highest as well. It is reasonable to assume that if the root cortex increases in more fertile habitats due to bigger cortex cells, the size of the stele is increasing as well.

Functional characteristics. Derived functional characteristics: RTD, SRA, SRL and SEA — calculated from direct measurements and reflecting site quality of spruce stands are more intimately related to the functioning of absorbing roots. 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 length or surface area, and root cost (construction and maintenance) is proportional to mass (Eissenstat and Yanai, 1997; Fitter, 1991; Lõhmus et al., 1989; Pregitzer et al., 2002).

The most important morphological parameters related to root functional status were the SRA and RTD of ectomycorrhizas; the SRA was larger and RTD was lower in highly productive spruce stands (**II**).

Specific root area of Norway spruce ECM has been recorded to vary from 28 to 63 m^2 per kg dry weight in Norway spruce (Abrazhko, 1973; 1985; Lõhmus and Oja, 1983; Lõhmus et al., 1989); in paper (**II**) the means of different sites varied from 29 to 42 m^2 kg^{-1}. An increase in SRA indicates a larger surface area per dry weight. Hence, the larger the SRA, the more effective the economy strategy of allocation of assimilates to the build-up short roots is.

RTD of short roots is connected to physiological activity, being smaller for young unsenescent roots. The mean RTD of spruce ECM varied from 310 to 540 kg m^{-3}, in other words by a factor of 1.7, in the investigated stands, and was higher in spruce ECM growing in low quality sites (Kuusnõmme, Pikasilla; Table 1; **II**). The ECM RTD variability within spruce stands was lowest in Kuusnõmme and Putkaste and highest in Vigala; for individual samples from all investigated stands the RTD varied from 180 to 680 kg m^{-3}(**II**). Also, Eissenstat and Yanai (1997) indicated that the RTD of fine roots is not a constant and its variation may be ecologically important. Wahl and Ryser (2000) measured RTD in herbaceous plants and came to a similar conclusion, that low RTD characterised plants of productive habitats, and a high RTD was typical in plants growing in unproductive environments.

Most frequently the fine root morphology is characterised by SRL — specific root length (the length to dry weight ratio; Comas et al., 2002; Olsthoorn, 1991; Pregitzer et al., 2002). Different species seem to have the same general strategy, the SRL is larger in sites of high productivity, hence greater SRL indicates better exploitation of soil by root mass unit. Mean SRL of ECM in our studies varied from 30.8 to 47.2 m g^{-1} (**II**), which is more than three times higher than SRL for the finest roots (<1 mm in diameter) found by Persson et al., (1998). The difference is most probably caused by inhomogeneity of the finest root fraction, both in morphology and anatomy. The finest root fraction consists of roots with primary and secondary structure. Pregitzer et al. (2002) found that, for the first three orders of roots in *Picea glauca* in North America, SRL values varied approximately from 20 to 40 m g^{-1}, the decrease of SRL with increasing root order was also established.

Total variation of derived characteristics includes a genetically-determined component and the changes that are most probably caused by abiotic and biotic environmental factors. Among biotic factors, mycorrhizal short roots are influenced by the fungal symbiont, since the proportions of the mantle in the root volume and in the root biomass vary with different ECM species (**V**). For example, the mean RTD of black ECM (mainly *Cenococcum*) compared to all other ECM was significantly smaller within the Roela study area (**II**). At the same time, the respective difference in SRA was insignificant, since an inverse relationship exists between RTD and D (14). The mean diameter of black ECM was significantly greater than the means of other ECMs in all cases.

Change in SRA is probably a way for plants to respond to the changes in environmental conditions (Lõhmus et al., 1989). Morphological studies in seven spruce stands showed that soil conditions had a significant impact on the morphology of short roots of Norway spruce. All measured soil variables explained 28% of total variance of root characteristics (**II**). The soil nutrient regime and, to a lesser extent, the soil water regime (described by the humus content, field capacity and specific soil surface area) were especially important. Similarly to SRA the values of SRL were also higher in high quality sites, since, when 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. In our stands the SRA was more sensitive to site quality than SRL.

Specific endodermal area is a strictly phytocentric characteristic, which was strongly related to site quality of spruce stands (Table 5); the parameter reflected site quality even better than SRA or RTD. SEA was larger in stands with higher productivity. Eissenstat and Achor (1999) reported that the number of passage cells was higher in thicker roots than in thinner roots. We can speculate that the larger SEA is, the better the mineral nutrition of ECM is.

Functional ECM characteristics (SEA, RTD, SRA, SRL) described the relation of ectomycorrhizas to site quality of spruce stands better than direct size measurements of short roots. The most sensitive parameter among functional characteristics was SEA, followed by RTD; the first increased and the second decreased towards optimal site conditions. According to Lõhmus (1984), the highest productivity of spruce is reached in the *Aegopodium* forest site type, followed by *Oxalis* and *Hepatica* forest site types. In our study, Väätsa stand belonged to the *Aegopodium* site type, but the stand productivity there was limited due to infection with a root rot fungus (*Heterobasidion annosum*). The resulting site deterioration was reflected in values of derived ECM parameters (RTD, SRA, SRL; **II**).

Relative fine root characteristics. At the level of single ECM root the proportions of different tissues express the functional efficiency of ECM. On the other hand, some proportional variables are more stable, being inherent to the plant species.

Single root level. The mean proportion of cortex in ectomycorrhizas' CSA varied significantly in the investigated spruce stands (59–67%), being the smallest in Putkaste, where the fungal mantle was significantly thicker. This indicates that the cortex and mantle, forming a transition zone between plant and fungus (cortex and Hartig net) and plant and soil (mantle and/or rhizodermal cells of cortex), have a significant, may be competitive impact on each other.

Our estimates of the share of fungal mantle in the volume of ECM in Norway spruce (from 18 to 28%; Table 3 in paper **V**) are close to the results obtained for Scots pine seedlings by Hobbie and Colpaert (2003), where the

proportion of fungal tissues in root biomass, estimated by ^{15}N budgeting, ranged from 12 to 22%.

The mean proportion of the stele in root CSA did not vary significantly in different stands (13–16%; Table 3 in paper V), hence it can be concluded that the parameter is relatively stable. Already Wilcox (1971) emphasized that fungal infection does not alter the basic structure of the stele. The stele CSA in total root CSA in herbaceous plants varied from 8.6 to 25.7% (Wahl and Ryser, 2000), and, as calculated on the basis of the results of Eissenstat and Achor (1999), from 12 to 18% for fibrous roots of citrus rootstocks. Most probably there exist species- or genera-related differences in the proportion of stele in root CSA.

When we excluded the fungal mantle, the mean CSA percentages of the cortex and stele were extremely stable (Table 3) and the ratio of cortex to stele on a CSA basis was 4 : 1. No differences between stands were revealed unless there were big differences in soil parameters and the site quality class varied between IV-V and I^a (Table 1). It can be concluded that, irrespectively of soil and site conditions, including the influence of fungal symbionts, the ratio of cortex to stele, 4 : 1 on a CSA basis, is inherent to Norway spruce.

On the basis of preliminary results for *Alnus incana* the respective proportion of cortex to stele of ECM was approximately 7 : 1. The smaller proportion of stele is in accordance with better xylem conductivity of deciduous trees compared to coniferous species. However, more measurements should be made to give an estimate of the cortex to stele ratio.

The stand level. The application of the commonly used definition of fine roots as those with <2 mm diameter for all tree species is problematic, at least in temperate forests, because it lumps together populations of roots that cycle carbon at significantly different rates (Gaudinski et al., 2001; Gill and Jackson, 2000; Pregitzer et al., 2002; Wells and Eissenstat, 2001). Roots have traditionally been separated into diameter classes of <1 mm and 1–2 mm. Fine roots of 1–2 mm in diameter mostly have a secondary structure, which is functionally significant for water and nutrient transport within the plant, and for tree stability (Coutts, 1983). More and more studies have been published where fine roots are separated not on a diameter basis, but from a functional aspect: mycorrhizas, conducting finest roots (<1mm), and fine roots (1–2 mm) (King et al., 2002). In some studies the roots were dissected by order with the distal roots numbered as first-, second- or third-order roots (Majdi et al., 2001; Pregitzer et al., 2002), or at least <1mm fine roots as finest roots and 1–2 mm (1–3 mm) fine roots as woody long roots (**VIII**; Cronan, 2003).

The ectomycorrhizas formed 39% of <1mm roots (calculated by mean root tip weight multiplying with tips number). The finest roots (<1 mm) formed 66% of the biomass of all the <2mm roots (Fig 7). We calculated, according to Cronan (2003), the mean share of <1mm roots in the <3mm root biomass to be 64% for a 55-year-old Norway spruce stand. The proportion of different

diameter classes was investigated in Roela in a 40-year-old stand (Lõhmus and Oja, 1983; Lõhmus et al., 1991; Table 1 in paper **VII**); the biomass of fine roots <1 mm was 1.3 t ha^{-1} and formed 66% of the <3 mm root biomass. In a deciduous forest, <1 mm roots constituted ~92% of the <2 mm root biomass (calculated from Fahey and Hughes, 1994).

The annual NPP of <1mm roots made up 73% of the annual NPP of <2 mm roots and this is also in good accordance with Cronan's (2003) findings, in which the NPP of <1 mm roots made up the greater share (71%) of the annual NPP of <3 mm roots.

The different shares of diameter fractions (<1 and 1–2 mm) of <2 mm root biomass and annual NPP have an effect on forest stand carbon cycling, as the turnover rate of <1 mm and 1–2 mm root diameter classes can be different (Table 7).

4.2. Fine root dynamics

Biomass and necromass dynamics of fine roots, assessed by soil cores and ingrowth cores.

Soil cores. The biomass of different fine root diameter classes, <1 mm and 1–2 mm, varied from 444 kg ha^{-1} (November) to 1315 kg ha^{-1} (October) and from 192 kg ha^{-1}(November) to 675 kg ha^{-1} (July and October), respectively, being significantly lower in November compared to other months (**VII**). The necromass of <1 mm and 1–2 mm roots varied from 1040 kg ha^{-1} (August) to 1765 kg ha^{-1} (October) and from 80 kg ha^{-1} (June 1996) to 280 kg ha^{-1} (July), respectively. The biomass of Norway spruce fine roots (d<2 mm) has been shown to range from 600 to 5750 kg ha^{-1} for spruce stands in Europe (Majdi and Persson, 1995; Sandhage-Hoffmann and Zech, 1993; Schmid and Kazda, 2002); our results remain within these limits (Fig 4). It has been reported in literature that both biomass and necromass of fine roots can fluctuate seasonally within a stand by as much as several times (Cronan, 2003; Keyes and Grier, 1981; Murach, 1987; Persson 1978; Vogt et al., 1981). The biomass of <1 mm roots varied seasonally 3, and necromass 1.7 times; for 1–2 mm roots the respective number was 3.5 for both bio- and necromass. However, the seasonal variability of different fine root diameter classes is considered in only a few cases. Functionally, the fine root fractions < 1 mm and 1–2 mm are different; the thicker fraction is more homogeneous and acts as a spreading, carrying and conducting system for short ECM roots. King et al. (2002) confirmed that fine roots of 1–2 mm diameter appeared to be part of the perennial root system with a higher life span, while the fine roots less than 1 mm in diameter were highly dynamic and with a shorter life span.

In 1996 and 1997, the share of living roots of the total (live+dead) root mass for diameter fractions of both <1 mm and 1–2 mm was significantly higher in

August and, for 1–2 mm roots, also in June (**VII**). Comparing the monthly values in Fig 5A and 5B to the annual mean, the biggest negative difference was observed in November. The proportion of living roots in the total (live+dead) fine root mass was closest to the annual mean in the soil core samples collected in September–October (Fig 5A and 5B), which is something that should be borne in mind if fine root samples for fine root biomass assessment in a stand are gathered only once a year.

The share of living finest roots in the total fine root mass depends both on biomass and necromass changes; the ratio live/(live+dead) increases when biomass increases and/or the necromass decreases. We found that in August a big part of necromass that was produced in July disappeared. Most probably, rapid decomposition of unsenescent roots occurred; it has been reported in literature that younger roots have a higher mortality risk and that this is related to seasonal factors (Wells et al., 2002). Generally, unsenescent root tips decompose quickly.

It has been proved that soil microbial communities are supported by assimilates from plants (girdling experiment) (Högberg et al., 2001; Näsholm et al., 1998). The possibility of the stimulation of decomposition by live roots has been discussed by Robinson et al. (1989); Lõhmus and Ivask (1995) showed that decomposing finest (<1 mm) roots had in the first two years taken additional energy from outside, most probably, via hyphae, from trees.

The fine root dynamics described above is inherent to natural soil conditions. Ingrowth cores artificially create a free soil volume, which enables the dynamics of its invasion to be observed, and it takes some time until the initial bio and necromass structure of fine roots is restored.

Ingrowth cores. The root growth dynamics in initially root-free soil volumes were different for long and short roots. The long roots of diameter 1–2 mm occupied ingrowth cores first; they constituted on average a half of all fine roots (<2 mm) at the end of the second year. In third year ingrowth cores, 1–2 mm roots formed approximately one third of <2 mm root biomass, which is based on soil core results inherent to the stand (Fig 4). Accordingly, the production of long roots in ingrowth cores was greater in the second than in the third year (Table 6). Woody roots of diameter 1–2 mm develop from long roots, with more absorbing roots growing on them later.

Total mass of <2 mm fine roots was 440 kg ha^{-1} at the end of the first year, and the share of dead roots was only 1,5%. In the second and third year, the mean share of dead roots in the total <2 mm root mass was 19% and 49%, respectively. The 3rd year value is close to that obtained by sequential coring, where dead roots formed 52% of the fine, d<2 mm, root mass. Comparison of the results obtained by the sequential soil coring and ingrowth core methods showed that in the third year after the insertion of the ingrowth cores the natural fine root structure disturbed by the insertion began to reestablish in the initially root-free soil.

Evaluation of methods used. As discussed above, the relative parameters of the 3rd year ingrowth cores already coincided with the results obtained by the soil core method, although both the fine root biomass and the annual NPP in third year ingrowth cores had not reached the same level. Makkonen and Helmisaari (1998) compared soil and ingrowth cores for estimating *Pinus sylvestris* fine root NPP and concluded that, during the third year, the Scots pine biomass production calculated by the ingrowth core method was similar to that calculated by the soil core method. Therefore, the local climate and soil conditions should be taken into consideration.

In paper **VII** we analysed two root research methods (soil and ingrowth cores) with regard to both the objectivity of root production estimation and labour intensity.

On the basis of the data from the two methods, we tried to find a combined method for determining the NPP of fine roots that would be less time consuming. Since the relative measures in ingrowth cores (Fig 4 and Tables 6, 7.) stabilised by the third year, the fine root production was calculated by multiplying the turnover rate of ingrowth cores by the fine root biomass estimated by soil coring (Fig 6). The suggested combined method gave an 11% higher estimate of fine-root-measured annual NPP for fine root biomass in October and an 8% smaller estimate for fine root biomass in September in our study stand. Thus, the combined method for estimation of fine root NPP gave good results and was less labour-intensive. The suitability of the combined method for wider application requires further analysis.

Vertical distribution in the undisturbed soil and in ingrowth cores. The highest rooting density was found in the upper part of the soil profile, where the concentration of nitrogen and organic matter as well as the activity of soil microbial communities were higher (Truu et al., 2001). The vertical distribution of the finest roots (<1 mm) tends to be more shallow than that of roots 1–2 mm in diameter; about 78% of <1 mm root biomass and about 61% of 1–2 mm root biomass were found to be located in the forest floor and the upper 10 cm mineral soil horizon. The similar vertical distribution of fine roots (<2 mm) in soil cores and ingrowth cores (Fig 3) demonstrates that root vertical distribution is primarily governed by the gradients of soil characteristics. Lõhmus et al., (1986) found that the vertical distribution of fine roots in a 60-year-old stand did not differ from that in a 40-year-old stand. In Estonian spruce stands, over 80% of <2 mm fine roots are located in the forest floor humus layer and in the topmost 20 cm mineral soil layer (Lõhmus and Lasn, 1990).

The share of living roots in the total fine root mass decreased with increasing soil depth in both the d<1 mm (from 53% in the forest floor to 16% at a depth of 30–40 cm) and d = 1–2 mm (from 71% to 42% in respective horizons) root fractions; this indicates the deterioration of soil conditions with increasing depth, which is typical of many soils. Our results are in good accordance with those of Strober et al. (2000), who found that the share of live fine roots in the total fine-root mass falls from 61% in the upper 0–5 cm layer to 22% at a depth

of 20–40 cm. Hence, the vertical distribution of the proportion of live fine roots most probably indicates the different vitality of the finest roots at various depths, because the impact of depth on fine root decomposition dynamics in the Roela study area was insignificant (**I**; Lõhmus and Ivask, 1995).

Turnover. Root turnover is an important sink for plant primary production. In the high productive Roela stand the root turnover in soil cores was 1.8 times the biomass of <2 mm roots, but, 1.4 times this biomass in ingrowth cores in the third year (**VII**). For the finest roots the turnover rate in soil cores and in third year ingrowth cores was similar (Table 7). Hence, the different turnover rate of <2 mm roots in soil cores and in 3rd year ingrowth cores is caused by the different turnover of thicker roots, 1–2 mm in diameter. The difference may probably partly be explained by differences in the vegetation periods. Soil cores were collected from June 1996 to June 1997, when the dominating meteorological conditions were similar to the average of many years. For the third year ingrowth cores the year 1999 was unfavourable: a cold early summer and severe drought from July to September. It should be pointed out that in ingrowth cores all roots, including the longer-living roots with a secondary structure, were younger than 3 years.

The fine root life span values (Table 7) are in good accordance with published minirhizotron data, in which the methodology used for calculating longevity of roots presents median root life span, not the average (Burton et al., 2000; King et al., 2002; Majdi et al., 2001). The comparability of the median and the calculated average root longevity is an open question, because there is no published work in which minirhizotron and soil core or ingrowth core methods have been used for estimating fine root longevity in the same location.

We compared our results to the corresponding published data for coniferous trees and found that there were significant differences in root life spans between sites (Table 7 in paper **VII**).

This table reveals the tendency for finer roots to have a shorter life span than thicker ones, but, on the other hand, the mycorrhizal infection increases the longevity of infected short roots (King et al., 2002). The fine roots of younger stands (Helmisaari et al., 2002) or of high productivity stands tend to live longer (Keyes and Grier, 1981). Fine root life span differs among species, ranging from < 20–1000 days (Eissenstat & Yanai, 1997; Black et al., 1998) according to the changes in plant ontogeny (Johnson et al., 2000), fine root diameter (Wells & Eissenstat, 2001), age, order and depth (Majdi et al., 2001; Wells et al., 2002), and in time-of-year and other factors (e. g. Eissenstat & Yanai, 1997; Johnson et al., 2000).

Mycorrhizas are obviously important modifiers of root turnover and should receive greater attention when estimating cycling of C and nutrients in forest ecosystems (Fogel, 1980). Turnover rate of most extramatrical mycelial structures estimated in mycelium in a soil microcosm appears to be a few weeks (Lindahl et al., 2002).

Decomposition. Root decomposition is a key process in the nutrient, mass and energy dynamics of a coniferous forest. The initially similar fine root (<2 mm) material had lost from 27% to 51% of its dry weight at eight study sites throughout Estonia after five years, and the decay rates were smaller at lower quality sites (Table 8). In different soils, after the first year fine spruce roots had lost from 21 to 33% of their initial dry weight, which is in good accordance with Berg and McGlaugherty (2003), who describe from 19% to 40%first year mass loss in boreal forests. The ash-free dry weight of the <1 mm root litter samples decreased by 14.3% during the first month. This is comparable with Cronan's (2003) results that the averaged decomposition rate for <1mm Norway spruce roots during the growing season is 11.5% per month.

In decomposition dynamics according to depth, no significant differences in the remaining mass of the finest (<1 mm) roots were found in the Norway spruce stand in Roela (paper **I**, Lõhmus and Ivask, 1995; Lõhmus et al., 1991), a result that can not be generalized to all habitats (Berg and McClaugherty, 2003). Comparing the decomposition rate of < 1mm and <2 mm roots in the same soil type (Roela and Voore 2), it appeared that during the first three years the decay rates of the finest (<1 mm) and fine (<2 mm) spruce roots were similar. On the other hand the amounts of soluble compounds in <2 mm roots were smaller than in the <1 mm root fraction (**I**). According to Swift et al (1979) fine root decomposition depends on substrate quality, decomposer communities, and environmental conditions. The impact of litter quality and environmental conditions have mainly been discussed in literature (Berg and McClaugherty, 2003). However, the impact of decomposer communities inherent to the site could be even more essential. In Pikasilla study area, fine-root litter decomposition of spruce and pine was compared; the decay rates were similar despite the initial N, ash and lignin contents being different (**I**); it means that decomposition rate depends mainly on the activity of decomposer communities. At the same time, the decomposer communities are interrelated with soil parameters that reflect the environmental conditions. Community-level physiological profiles of soil microbial samples (includes the spruce stands at Roela and Kuusnõmme and the grey alder stand in Porijõgi) were correlated with the gradient of organic matter and nitrogen, and the second most important factor was soil pH value (Truu et al., 2001). The earthworm community is one of the most important members of the soil fauna attending in the regulation of decomposition and nutrient cycling (Edwards and Bohlen, 1996). In spruce forests, the numbers of earthworm individuals and species were highest in study area with the highest productivity (Roela, named Voore in paper **III**). Hence, in productive forest sites, more active decomposer communities exist compared to poorer sites.

4.3. NPP allocation in a Norway spruce forest ecosystems

The calculated estimate of fine root (<2 mm) NPP in the 40-year-old Roela stand was 2.8 t ha^{-1} yr^{-1} and the respective share of fine roots in the net primary production of the stand was around 13% (Fig 7). This value is relatively small compared to the results revealed in most studies, and the reason is most probably related to favourable soil conditions (**VII**). In literature, fine roots constituted 8 to 76% of the annual total NPP (Fogel 1985; Grier et al., 1981; Gower et al., 1996; Helmisaari et al., 2002; Keyes and Grier, 1981; Nadelhoffer and Raich, 1992; Persson, 1993). Gower et al. (2001) tried to summarize NPP and carbon allocation patterns for boreal forests in relation to climatic and biological variables, based on knowledge obtained from SWECON and BOREAS; fine roots accounted for 19 to 50% of the total NPP (Gower et al., 2001). However, according to SWECON, the C flux into the belowground system via the tree stem was 63% of net photosynthetic production (Ågren et al., 1980). Högberg et al. (2002) stated that the C allocation from the NPP to the root system is overestimated and that this raises many questions: how do the assimilates become divided belowground within roots and microbial associations (including ectomycorrhizal fungi), how to separate the respiration of roots and microbial communities. We found that the activity and diversity of microbial communities was always significantly higher in the soil-root interface than in bulk soil for both species, Norway spruce and grey alder (Truu et al., 2001), which indicates the support of rhizosphere microbes by trees.

Keyes and Grier (1981) compared the proportion of fine roots in the total NPP of Douglas firs for two sites: low and high productivity, the results were 36.4% and 7.9%, respectively. This is in accordance with the general concept that in unfavourable site conditions more NPP is allocated to roots (Olsthoorn, 1991; Palumets, 1991; Vogt et al., 1987). In a fertile site the trees compete mostly for light, but in a poor site, for water and nutrients (Köstler et al., 1968).

The decrease in the proportion of fine roots in NPP can be attributed to some extent to variations in short root morphological and anatomical features leading to higher functional efficiency (**II**, **V**).

Fungal biomass in fine roots. The carbon cost of fungal or bacterial symbionts in root systems should be included as part of field estimates of belowground production, but is not, because of sampling difficulties (Vogt et al., 1998). Data on how much fungal tissues incorporated in ectomycorrhizas contribute to biomass for field grown tree roots, and information on the carbon cost of the fungal partner incorporated into ECM tissues are still scarce (Hobbie and Colpaert, 2003; Lindahl et al., 2002; Rousseau and Reid, 1989).

We have made an attempt to scale fungal biomass in fine roots to the ecosystem level. The mean mass of one ectomycorrhizal tip and the number of tips per ha were estimated. Accordingly, as the mean tip mass was 0.079 ± 0.007 mg and the mean number of living tips was $4.5 \times 10^9 \pm 0.5 \times 10^9$ per ha,

the mean mass of ectomycorrhizas per ha is calculated by multipling the values; the result is 355 kg. The proportion of the fungal mantle of an ectomycorrhizal root was estimated at 18% in this study area (**V**). Making the obviously simplifying assumptions that 1) the share of fungal mantle does not change between vegetation periods, 2) the tissue density within ectomycorrhizas is homogeneous, 3) carbon cost for a mass unit of plant and fungus in ectomycorrhizas is equal, we can estimate the share of fungal biomass in fine root biomass and NPP. The biomass of fungus is (18% × 355 kg)/100% = 64 kg. Ectomycorrhizas formed, on average, 39% of the finest (<1mm) roots. If the annual NPP of finest roots (<1 mm) is 1830 kg ha^{-1} yr^{-1}, then, proportionally, that makes about 714 kg ha^{-1} yr^{-1} for ectomycorrhizas, and accordingly 18%, or about 129 kg ha^{-1} yr^{-1}, is used up for the ectomycorrhizal mantle. These calculations are very rough, since, first of all, we have made some very audacious assumptions and, secondly, we have not taken into account the intraspecific variations in the ecyomycorrhiza-forming fungi. Since mycorrhizal fungi vary in growth rate and tissue quality, these changes in species assemblages could produce unforeseeable impacts on the productivity, survivorship, or decomposition of mycorrhizal biomass (Treseder and Allen, 2000).

4.4. Fine root parameters and site optimality

Fine root anatomical and morphological parameters were estimated and analysed on automorphic soils in the natural distribution area of Norway spruce in Estonia, which enabled the respective root characteristics to be related to the optimality of site and soil conditions (**II**, **V** and **VII**; discussed above). Maximum productivity is usually considered to be the main criterion of optimality. The site quality was highest in Roela (Table 1). In Roela, the highest number of earthworm individuals and species were also found and the high activity of microbial communities was established (**III**). Root related parameters indicating site optimality were considered at different organizational levels.

Cell level. The thickness of the cortex of Norway spruce ECM depended mainly on cell size, because the number of cell rows in the cortex did not vary between stands. The thickness of the cortex increased with increasing site productivity (**II**).

Tissue level. On the tissue level there was a tendency for the thickness of the cortex and diameter of the stele to increase with increasing site productivity (**II**). There was no such trend in the thickness of the fungal mantle among the analysed spruce stands; this indicates the autonomous behaviour of the fungal symbiont.

Organ level. The functional parameters of ECM — SRA, SEA and SRL increased, and RTD decreased, towards optimal site conditions; the means that ECM diameter tended to increase with increasing site quality (**II** and **V**).

Tree as organism level. The root system of coniferous trees may generate millions of root tips each with its own meristem (Sutton, 1980). In Roela, the mean number of ectomycorrhizal tips was 4.3 million per tree (**VII**). Kalela (1954) found that the root system of one 100-year-old Scots pine possessed 5 million root tips.

Forest ecosystem level. Most comparative studies (Keyes and Grier, 1981; Schmid and Kazda, 2002; Vogt et al., 1983) have shown a trend of lower fine root mass in nutrient rich compared to nutrient poor soils, where a smaller proportion of fine roots is necessary for sufficient nutrient supply. Considering the results obtained for Norway spruce in Estonia, the mean share of <1 mm root biomass in the total belowground biomass was 2.6% in a high productivity Norway spruce stand (Lõhmus and Oja, 1983) and 4.3% in a low productivity stand (Palumets, 1991).

As discussed above, the share of fine roots in NPP (13%) was among the lower values quoted in literature. The mean number of ectomycorrhizal root tips per basal area of stand (m^2) was 90×10^6 in Roela. The same parameter was estimated for two high quality stands (Voore: 86×10^6 and Väätsa: 80×10^6) and one low site quality stand (Pikasilla: 170×10^6) by Lõhmus and Lasn (1990), and from this we can conclude that the number of ECM roots formed per basal area is higher in stands with low site quality, and falls with improving site quality. Keyes and Grier (1981) also reported that the rate of root tip production was 2.5-fold greater in the low productivity site.

5. CONCLUSIONS

The functional efficiency of absorbing roots of Norway spruce increases as site conditions are improved, and root NPP forms an essentially smaller share of the stand NPP than the above-ground production. In the investigated high productivity Norway spruce stand the fine root (<2 mm) net primary production was 2.5 t ha^{-1} yr^{-1}, which accounted for around 13% of the net primary production. The obtained value is one of smallest reported in published studies; the reason is most probably related to favourable soil conditions. In favourable site conditions the activity and diversity of decomposer communities was higher and fine root decomposition, faster.

Functional short root characteristics (SEA, RTD, SRA, SRL) described the relation to site optimality of Norway spruce stands better than direct size measurements of short roots. The most sensitive parameter among functional characteristics was SEA, followed by RTD; the first increased and the second decreased as optimal site conditions were approached.

The morphological variability of Norway spruce short roots is related to soil conditions. The soil nutrient regime and, to lesser extent, the soil water regime (described by the humus content, field capacity and specific soil surface area) explained 28% of the total variability.

All short roots of Norway spruce in Estonian study areas were ectomycorrhizas. Concerning the ECM anatomy, the plant and fungal components of Norway spruce ECM behaved autonomously and the quality of site conditions is reflected in the dimensions of the plant tissues. The thickness of the cortex and, to a lesser extent, the diameter of stele increased as optimal site conditions for Norway spruce were approached. For the thickness of fungal mantle of ECM roots there was no correlation with site quality class. The thickness of the cortex of Norway spruce ECM depends mainly on cell size, because the number of cell rows in the cortex did not vary between stands. Accordingly, it can be concluded that irrespectively of soil and site conditions, including the influence of fungal symbionts, the proportion of cortex to stele, 4: 1 on ectomycorrhiza CSA basis, is inherent to Norway spruce.

Comparing two different methods (sequential cores and ingrowth cores) used to estimate the fine root bio- and necromass, NPP, turnover rates, and longevities, the mass and NPP values for 3rd year ingrowth cores were approximately two times smaller than those obtained by soil cores. In the studied area, soil cores and 3rd year ingrowth cores produced turnover rates and longevities of fine roots (<2mm) that were similar. For both methods the biomass and NPP of finest roots (<1 mm) formed approximately two thirds of that of <2 mm roots. The turnover rate for finest roots was approximately 2.0 times the biomass, and longevity was half a year. The estimates remain within the bounds of the published minirhizotron measurements.

The more detailed classification of fine roots (diameter classes <1 mm and 1–2 mm in this work) enables a more thorough understanding of the fine-root production process. Our results clearly showed the differences in fine root vertical distribution, growth pattern in root-free soil, and the amount of biomass and annual NPP, between roots separated by diameter into classes <1mm and 1–2 mm. In further investigations, the differentiation of fine roots based on their function should be taken into consideration.

Fine-root biomass was stable during the vegetation period. According to our results fine root biomass may be measured in any month in the vegetative period, nevertheless, in the given climatic conditions, the biomass tended to be smaller in June, when the detrimental effects of winter cannot be ruled out and the intensive allocation of assimilates to growing shoots is in progress. The fine root biomass decreased after the end of the vegetation period, in Estonian climatic conditions, in November. Fine root biomass was 3 times lower in November than in October.

From the methodological point of view it is essential to use absolute and relative measures from the absorbing-root scale through to the stand scale in order to better express the functional efficiency of the forest ecosystem. In addition, the relative root parameters enable different root research methods to be combined and less labour-intensive root study methods to be derived. The new combined method based on turnover rate of 3rd year ingrowth cores and fine root biomass estimated by soil cores gave acceptable results in the investigated stand and was less labour-intensive than sequential coring. The suitability of the combined method for wider application requires further analysis.

6. REFERENCES

Aber JD, Melillo JM, Nadelhoffer KJ, McClaugherty CA, Pastor J. 1985. Fine root turnover in forest ecosystems in relation to quantify and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317–321.

Abrazhko MA. 1973. Vsasyvayushchaya poverkhnost' kornej (Absorbing surface of roots). *In* Struktura i produktivnost' elovykh lesov yuzhnoj taigi (Structure and productivity of spruce forests.) Ed. V G Karpov. pp 132–133. Nauka, Leningrad.

Abrazhko MA 1985 O vliyanij azotnykh udobrenij na raspredelenie I fraktsionnyi sostav kornej *Picea abies (Pinaceae)* (The effect of nitrogen fertilizers on spatial distribution and fractional composition of *Picea abies (Pinaceae)* roots). Botanitcheskij zhurnal (Soviet Botanical Journal) 70, 250–254.

Agerer R. 1987–1997. Colour Atlas of Ectomycorrhizae. 1st–8th edn. Einhorn-Verlag, Swäbisch Gmünd, Germany.

Ågren GI, Axelsson B, Flower-Ellis JGK, Linder S, Persson H, Staaf H, Troeng E. 1980. Annual carbon budget for a young Scots pine. Ecol Bull 32, 307–313.

Ahlström K, Persson H, Börjesson I. 1988. Fertilization in a mature Scots pine (*Pinus sylvestris* L.) stand — effects on fine roots. Plant Soil 106, 179–190.

Allen MF. 1991. The ecology of ectomycorrhizae. Cambridge, UK: Cambridge University Press.

Arthur MA, Fahey TJ. 1992. Biomass and nutrients in an Engelmann spruce-subalpine fir forest in north central Colorado: pools, annual production and internal cycling. Can J For Res 22, 315–325.

Berg B, McClaugherty C. 2003. Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. 283p. Springer

Black KE, Harbron CG, Franklin M, Atkinson D, Hooker JE. 1998. Differences in root longevity of some tree species. Tree Physiol 18, 259–293.

Brady NC. 1990. The Nature and Properties of Soils. MacMillan Publishing Company, New York. 621p.

Burton AJ, Pregitzer KS, Hendrick RL. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125: 389–399.

Bücking H, Heyser W. 2001. Microautoradiographic localization of phosphate and carbohydrates in mycorrhizal roots of *Populus tremula* × *Populus alba* and the implications for transfer processes in ectomycorrhizal associations. Tree Physiology 21, 101–107.

Comas LH, Bouma TJ, Eissenstat DM. 2002. Linking root traits to potential growth rate in six temperate tree species. Oecologia 132, 34–43.

Coutts MP. 1983. Development of the structural root system of Sitka spruce. Forestry 56, 1–16.

Cronan CS. 2003. Belowground biomass, production, and carbon cycling in mature Norway spruce, Maine, U.S.A. Can. J. For. Res. 33, 339–350.

Edwards CA, Bohlen PJ. 1996. Biology and Ecology of Earthworms. 3rd edition. London, Chapman & Hall, 426 pp.

Egli S, Amiet R, Zollinger M, Schneider B. 1993. Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. Trees 7, 123–129.

Eissenstat DM, Achor DS. 1999. Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytol. 141, 309–321

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

Eissenstat DM, Yanai RD. 2002. Root Life Span, Efficiency, and Turnover. *In*: Y. Waisel, A. Eshel, U. Kafkafi (Eds.) Plant Roots: The Hidden Half. Third edition. Marcel Dekker, New York., 221–238.

Ekblad A, Wallander H, Carlsson R and Huss-Danell K 1995 Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. New Phytol. 131, 443–451.

Enstone DE, Peterson CA and Hallgren SW. 2001. Anatomy seedling tap roots of loblolly pine (Pinus taeda L.). Trees 15, 98–111.

Erland S and Taylor AFS. 2002. Diversity of Ecto-mycorrhizal Fungal Communities in Relation to the Abiotic Environment. *In* Mycorrhizal Ecology. Ecological Studies, Vol. 157. Eds. M G A van der Heijden and I Sanders. pp 163–200. Springer-Verlag, Berlin Heidelberg.

Fahey TJ, Hughes JW. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. J Ecol 82, 533–548.

Fairley RI, Alexander IJ. 1985. Methods of calculating fine root production in forests. In: Fitter AH (ed.). Ecological interactions in soil. Spec. Publ. Br. Ecol. Soc. 4, 37–42.

FAO — Unesco. 1988. Soil map of the world. Revised Legend. World Soil Resources Report 60, Rome, 119 p.

Fitter AH. 1985. Functional significance of root morphology and root system architecture. *In* Ecological Interactions in Soil. Special publication of the British Ecological Society, No 4. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 87–106. Blackwell Scientific, Oxford, UK.

Fitter AH. 1991. Characteristics and functions of root systems. In: Waisel Y, Eshel A, Kafkafi U (Eds.). Plant roots — the hidden half. Marcel Dekker, New York, 3–24.

Fogel R. 1980. Mycorrhiza and nutrient cycling in natural forest ecosystem. New Phyto 86, 199–212.

Fogel R. 1985. Roots as primary producers in below-ground ecosystems. *In* Ecological Interactions in Soil. Special publication of the British Ecological Society, No 4. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 23–36. Blackwell Scientific, Oxford, UK.

Frey T. IBP research at Vooremaa Forest Ecology Station. *In* Spruce forest ecosystem structure and ecology I. Estonian Contributions to the IBP No 11. Tartu, 21–36.

Gaudinski JB, Trumbore SE, Davidson EA, Zheng S. 2000. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. Biochemistry 51, 33–69.

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD. 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. Oecologia 129, 420–429.

Gill RA, Jackson R 2000 Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147, 13–31.

Gower ST, Vogt KA, Grier CC. 1992. Carbon dynamics of Rocky mountin Douglas-fir: influence of water and nutrient availability. Ecol Monogr 62, 43–65.

Gower ST, Isebrands JG, Sheriff DV. 1995. Carbon allocation and accumulation in conifers. In: Smith WK, Hinckley TM (Eds.), Resource Physiology of Conifers. Academic Press, San Diego, CA, 217–254.

Gower ST, McMurtrie R, Murty D. 1996. Above-ground net primary production decline with stand age: potential causes. Trees 11, 378–382.

Gower ST, Krankina O, Olson RJ, Apps M, Linder S, Wang C. 2001. Net primary production and carbon allocation patterns of boreal forest ecosystems. Ecol Appl 11 (5), 1395–1411.

Grogan P, Baar J, Bruns TD. 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology 88, 1–13.

Grier CC, Vogt KA, Keyes MR, Edmonds RL. 1981. Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. Can. J. For. Res. 11, 155–167.

Harley JL, Smith S. 1983. Mycorrhizal Symbiosis. Academic press, London, 481p.

Helmisaari HS, Makkonen K, Kellomäki S, Valtonen E, Mälkonen E. 2002. Below- and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. Forest Ecology and Management 165, 317–326.

Hendrick RL, Pregitzer KS. 1993. The dynamics of fine root length, biomass and nitrogen content in two northern hardwood ecosystems. Can J For Res 23, 2507–2520.

Hobbie EA, Colpaert JV. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol. 157, 115–126.

Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10, 1855–1871.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.

Högberg P, Nordgren A, Ågren GI. 2002. Carbon allocation between tree growth and root respiration in boreal pine forest. Oecologia 132, 579–581.

Jackson RB, Mooney HA, Schulze ED. 1997. A global budget of fine root biomass, surface area, and nutrient contents. Proc Natl Acad Sci USA 94, 7362–7366.

Jones RH, Mitchell RJ, Stevens GN, Pecot SD. 2003. Controls of fine root dynamics across a gradient of gap sizes in a pine woodland. Oecologia 134, 132–143.

Johnson MG, Philips DL, Tingey DT, Storm MJ. 2000. Effects of elevated CO_2, N-fertilization, and season on survival of ponderosa pine roots. Can J For Res 30, 220–228.

Jonsson L, Dahlberg A, Nilsson M-C, Kåren O, Zackrisson O. 1999. Continuity of ectomycorrhizal fungi in self-regenerating boreal forests of *Pinus sylvestris*: comparative analysis of diversity of mycobionts of seedlings and old trees. New Phytol. 142, 151–162.

Kalela E, 1954. Mäntysiemen puiden ja puustojen juurisuhteista. Acta For Fenn 61, 1–17.

Keyes MR, Grier CC. 1981. Above- and belowground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can J For Res. Vol. 11, 599–605.

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherry P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol. 154, 389–398.

Kõlli R, Kährik R. 1970. Biomass and its growth of *Fragaria-Hepatica* forest type. (In Russian). Sb, nauch. Tr. Estonskoi selskohosjaistvennoi akademii 65, 69–91.

Kutschera L, Lichtenegger E. 2002. Wurzelatlas mitteeuropäischer Waldbäume und Sträucher. 6. Band der Wurzelatlas-Reihe. Leopold Stocker Verlag, Graz-Stuttgart, 604 S.

Köstler JN, Brückner E, Bibelriether H. 1968. Die Wurzeln der Waldbäume. Untersuchungen zur Morphologie der Waldbäume in Mitteleuropa. Verlag Paul Parey, Hamburg und Berlin, 107–111.

Leuschner C, Hertel D, Schmid, Koch O, Muhs A, Hölscher D. 2003. Stand biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil (in press).

Lindahl BO, Taylor AFS. Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests — towards a less 'phytocentric' perspective. Plant Soil 242, 123–135.

Lõhmus, E. 1984. Eesti metsakasvukohatüübid. Tln. 88 lk.

Lõhmus K, Oja T. 1983. K metodike izuchenja podzemnoi chasti drevostoev. (The methodology of studying below-ground part of stands.) (In Russian) Lesovedenie 4., 56–62.

Lõhmus K, Lasn R, Oja T. 1986. Rost kornej eli evropejskoj v zavisimosti ot pochvennykh uslovij. Pochvovedenie No 6. M., 89–97.

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

Lõhmus K, Lasn R 1990 Spruce and pine root structures and chemical characteristics in moderate acid soils. Air Pollution Research Report 32, 74–78.

Lõhmus K, Lasn R, Oja T. 1991. The influence of climatic and soil physical conditions on growth and morphology of Norway spruce roots. In: Plant roots and their environment. McMichael, B. L. and Persson, H. (eds.), Elsevier Science Publishers B. V., 233–239.

Lõhmus K, Ivask M. 1995. Decomposition and nitrogen dynamics of fine roots of Norway spruce (*Picea abies* (L.) Karst.) at different sites. Plant Soil 168–169.

Lõhmus K, Ivask M, Ostonen I. 1995. Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils. *In*: The Finnish Forest Research Institute. Research Papers 537. *Eds*. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83–87.

Lõhmus K, Mander Ü, Tullus H, Keedus K. 1996. Productivity, buffering capacity and resources of grey alder forests in Estonia. In: Perttu K, Koppel A (Eds). Short rotation willow coppice for renewable energy and improved environment. Uppsala, 95–105.

Luxova M. 1992. Root structure. Primary cortex. *In* Physiology of plant root system. Eds. J Kolek and V Kozinka. pp 52–59. Kluwer Academic Publishers, Dordrecht.

Majdi H, Persson H. 1995. A study on fine-root dynamics in response to nutrient applivations in a Norway spruce stand using the minirhizotron technique. Z Pflanzenernähr Bodenk 158, 429–433.

Majdi H, Nylund JE. 1996. Does liquid fertilisation affect life span of mycorrhizal short roots and fine root dynamics? Plant Soil 185, 305–309.

Majdi H, Kangas P. 1997. Demography of fine root in response to nutrient applications in a Norway spruce stand in south-western Sweden. Ecoscience 4, 199–205.

Majdi H, Damm E, Nylund J-E. 2001. Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytol. 150, 195–202.
Makkonen K, Helmisaari HS. 1998. Seasonal variation of fine-root biomass in a Scots pine (*Pinus sylvestris* L.) stand. For Ecol Manage 102, 283–290.
Makkonen K, Helmisaari HS. 1999. Assessing Scots pine fine-root biomass — comparison of soil core and root ingrowth core methods. Plant Soil 210, 43–50.
Malhi Y, Baldocchi DD, Jarvis PG. 1999. The carbon balance of tropical, temperate and boreal forests. Plant Cell Envir 22, 715–740.
Mander Ü, Kuusemets V, Lõhmus K, Mauring T. 1997. Efficiency and dimensioning of riparian buffer zones in agricultural catchments. Ecological Engineering, 8, 299–324.
Melillo JM, McGuire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL. 1993. Global climate change and terrestrial net primary production. Nature 363, 234–240.
Murach D. 1987. Judgement of the applicability of liming to restabilise forest stands with special consideration of root ecological aspects. Air Pollution and Ecosystems. Proceedings of an International Symposium in Grenoble, France 18–22 May 1987, pp 445–451.
Nadelhoffer KJ, Aber JD, Melillo JM. 1985. Fine-roots, net primary production and soil nitrogen availability: a new hypothesis. Ecology 66 (4), 1377–1390.
Nadelhoffer KJ. 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystem. New Phytol 147, 131–139.
Nadelhoffer KJ, Raich JW. 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. Ecology 73, 1139–1147.
Näsholm T, Ekbald A, Nordin A, Giesler R, Högberg M, Högberg P. 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916.
Nordström S, Rundgren S. 1973. Associations of lumbricids in Southern Sweden. Pedobiologia 13, 301–326.
Nye P H 1973 The relation between radius of a root and its nutrient absorbing power (α). J. Exp. Bot. 24, 783–786.
Olsthoorn AFM 1991 Fine root density and root biomass of two Douglas-fir stands on sandy soils in The Netherlands: 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth. J. Agric. Sci. 39, 1, 61–77.
Olsthoorn AFM, Tiktak A. 1991. Fine root density and root biomass of two Douglas-fir stands on sandy soils in the Netherlands. 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth J Agric Sci 39, 61–77.
Orlov AJa. 1957. Observations on absorbing roots of spruce (Picea exelsa Link) in natural conditions. Office of Technical Services, U. S. Department of Commerce, Springfield (In Russian) No. 60-21109. pp 15. Bot. Ž. 42 (8), 1172–1181.
Palumets J. 1988. Partitioning of phytomass in Norway spruce in relation to stand age and climate factors. (In Russian) Lesovedenie 2, 34–40.
Palumets J. 1990. Kui palju on kuusel juuri. EL. No 8, 512–515.
Palumets J. 1991. Analysis of Phytomass Partioning in Norway Spruce. Scripta Botanica VIII. Trt. 95 p.
Palumets J. 1995. Madalaboniteedilise kuuse massijaotuse analüüs. Lõhmus K (toim) Juureökoloogia seminar. Tartu 1995. 48–50.
Persson H. 1978. Root dynamics in a young Scots pine stand in Central Sweden. Oikos 30: 508–519.

Persson, H. 1980. Death and replacement of fine-root in a mature Scots pine stand. Ecol Bull 32, 251-260.
Persson HÅ. 1983. The distribution and productivity of fine roots in boreal forests. Plant Soil 71, 87-101.
Persson, H. 1993. Factors affecting fine root dynamic of trees. Suo 43, 163–172.
Persson H, Ahlström K, Clemensson-Lindell A. 1998. Nitrogen addition and removal at Gårdsjön — effects on fine-root growth and fine-root chemistry. For. Ecol. Manag. 101, 199–205.
Pregitzer KS, Hendrick RL, Fogel R. 1993. The demography of fine roots in response to patches of water and nitrogen. New Phytol 125, 575–580.
Pregitzer KS, Zak DR, curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytol 129, 579–585.
Pregitzer KS, Deforest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine North American trees. Ecological Monographs 72: 293–309.
Peter M, Ayer F, Egli S. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytol. 149, 311–325.
Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine North American trees. Ecological Monographs 72, 93–309.
Puri B, Murari K. 1964. Studies in surface-area measurements of soils. 2 Surface area from a single point on the water isoterm. Soil Sci. 97, 341–343.
Read, D. 1997. Mycorrhizal fungi: The ties that bind. Nature 388, 517–518.
Robinson D, Rorison I H. 1983. Relationships between root morphology and nitrogen availability in a recent theoretical model describing nitrogen uptake from soil. Plant Cell Environ. 6, 641–647.
Robinson D, Bryan G, Karl R, Wheatley R. 1989. Root-induced nitrogen mineralization. Plant Soil 117, 185–193.
Rousseau JVD, Reid CPP. 1989. Measurement of Carbon Cost in Ectomycorrhizae. *In* Applications of Continuous and Steady-State Methods to Root Biology. Eds. J G Torrey and L J Winship. pp 183–196. Kluwer Academic Publishers, Dordrech.
Sandhage-Hoffmann A, Zech W. 1993. Dynamik und element-gehalte von fichtenwurzeln in kalkgesteinböden am wank (Bayerishe Kalkalpen). Zeitschr. F. Pflanzenernährung und Bodenk. 156 (2), 181–190.
Schmid I, Kazda M. 2002. Root distribution of Norway spruce in monospecific and mixed stands on different soils. Forest Ecology and Management 159, 37–47.
Smith SE, Read DJ. 1997. Mycorrhizal Symbiosis, 2nd ed. (San Diego, CA: Academic Press).
Schnürer J, Rosswall T. 1985. Fluorescein hydrolysis as a measure of total microbial activity in the soil and litter. Appl Env Microbiol 43, 1256–1261.
Steele SJ, Gower ST, Vogel JG, Norman JM. 1997. Root mass, net primary production, and turnover in aspen, jack pine, and black spruce forests in Saskatchewan and Manitoba, Canada. Tree Physiol 17, 577–587.
Strober C, Eckart GA, Persson H. 2000. Root growth and response to nitrogen. In: Schulze E-D, ed. Carbon and Nitrogen Cycling in European Forest Ecosystems, Ecol Stud 142. Berlin; Springer, pp 99–121.
Sutton RF. 1980. Root system morphogenesis. New Zeal J For Sci 10 (1), 264–292.

Söderström BE, Bååth E. 1978. Soil microfungi in three Swedish coniferous forests. Hol. Ecol. 1, 62–72.

Swift MJ, Heal OW, Anderson JM. 1979. Decomposition in terrestrial ecosystems. Studies in ecology, Vol 3. Blackwell Sci Publ., 1979, 372 pp.

Ter Braak CJF. 1987. CANOCO — a FORTRAN Program for Canonical Community Ordination by [Partial] [Detrended] [Canonical] Correspondence Analysis. Principal Components Analysis and Redundancy Analysis (Version 2.1). 95 p. Agriculture Mathematics Group, Wageningen.

Treseder KK, Allen MF. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. New Phytol 147, 189–200.

Tullus H, Keedus K, Uri V, Mander Ü, Lõhmus K. 1996. Sustainable forests management in Estonia. Planning and Implementing Forest Operations to Achieve Sustainable Forests. USDA Forest Service Worth Central Forest Experiment Station. General Technical Report NC 186, 99–101.

Truu J, Truu M, Lõhmus K, Ostonen I, Ivask M, Kanal A. 2001. Structure and activity of microbial communities in soil-root interface and bulk soil in coniferous and deciduous stands. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root, 402–403.

Uri V, Tullus H, Lõhmus K. 2002. Biomass production and nutrient accumulation in short-rotation grey alder (Alnus incana (L.) Moench) plantation on abandoned agricultural land. Forest Ecology and Management, 161: 169–179.

Vogt KA, Edmonds RL, Grier CC. 1981. Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23- and 180-year-old subalpine Abies amabilis stands. Can J For Res. 11: 223–229.

Vogt KA, Grier CC, Meier CE, Keyes MR. 1983. Organic matter and nutrient dynamics in forest floors of young and mature Abies amabilis stands in Western Washington, as suggested by fine-root input. Ecol. Monogr. 53, 139–157.

Vogt KA, Grier CC, Vogt DJ. 1986. Production, turnover and nutritional dynamics of above- and belowground detritus of world forests. Adv Ecol Res 15, 303–307.

Vogt KA, Vogt DJ, Moore EE, Fatuga MB, Redlin MR, Edmonds RL. 1987. Conifer and angiosperm fine-root biomass in relation to stand age site productivity in Douglas-fir forests. J. Ecol. UK, 75, 857–870.

Vogt KA, Persson H. 1991. Measuring growth and development of roots. *In:* Techniques and Approaches in forest tree ecophysiology. Lassoie JP, Hinckley ThM (Eds). CRC Press, Boca Raton FL, 1991, 599 pp.

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H. 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187, 159–219.

Vogt KA, Vogt DJ, Bloomfield J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200, 71–89.

Wahl S, Ryser P. 2000. Root tissue structure is linked to ecological strategies of grasses. New Phytol. 148, 459–471.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. Ecology 82, 882–892.

Wells CE, Glenn DM, Eissenstat DM. 2002. Changes in the risk of fine-root mortality with age: a case study in peach, Prunus persica (Rosaceae). Am J Bot 89 (1): 79–87.

Wilcox HE. 1971. Morphology of Ectendomycorrhizae in *Pinus resinosa*. *In* Mycorrhizae. Ed. E Hacskaylo. pp 54–68. Proceedings of the first North American conference on mycorrhizae, April 1969. Misc. Publication 1189 U. S. Department of Agriculture, Forest Service, Washington.

Wilson PJ, Thompson K, Hodgson JG. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol 143, 155–162.

Yin X, Perry JA, Dixon RK. 1989. Fine-root dynamics and biomass distribution in a Quercus ecosystem following harvesting. For Ecol Manage 27, 159–177.

Yearbook Forest 2001. Aastaraamat Mets 2001. Keskkonnaministeerium, Metsakaitse- ja metsauuenduskeskus.Tartu 2002. 142 p.

7. SUMMARY IN ESTONIAN

Peente juurte struktuur, dünaamika ja osatähtsus kuusiku netoproduktsioonis sõltuvalt kasvukohatingimustest

KOKKUVÕTE

Metsaökosüsteemide aineringe probleemid on viimase veerandsajandi jooksul muutunud väga aktuaalseks, kusjuures üha suuremat tähelepanu on pööratud puistute maa-aluses osas toimuvatele protsessidele. Juurte dünaamikal on metsade aineringes väga oluline osa. Kuna juurte uurimine on paljudel erinevatel põhjustel raskendatud, on neist maapealse osaga võrreldes ka vähem teada. Möödunud sajandi viimase paarikümne aasta vältel leidis kinnitust seisukoht, et peente juurte osatähtsus metsade netoproduktsioonis võib olla olulisim. Leiti, et juurtesse võib minna üle 50 protsendi metsade aastasest produktsioonist (Ågren *et al.*, 1980; Grier *et al.*, 1981; Helmisaari *et al.*, 2002; Persson, 1980, 1983; Steele *et al.*, 1997;). Hiljutised tööd väidavad, et peente juurte netoproduktsioon on ülehinnatud ning hingamiseks kuluv osa alahinnatud (Högberg *et al.*, 2002). Teravalt on esile kerkinud ka juurte osa mulla mikroobikoosluste hingamise toetamisel ning selle tõttu on asutud kriitiliselt üle vaatama metsaökosüsteemi süsinikubilanssi (Högberg *et al.*, 2001, 2002).

Kirjandusest võib leida terve rea viimaste aastakümnete jooksul ilmunud töid, kus käsitletakse puude maa-alust osa, sealhulgas ka peente juurte produktsiooni, erakordselt suuri edusamme selles vallas on tehtud Põhjamaades, (Cronan, 2003; Helmisaari *et al.*, 2002; Makkonen ja Helmisaari, 1998, 1999). Nii maapealse kui ka maa-aluse osa produktsiooni, sealhulgas allokatsiooni, analüüsivaid töid on endiselt vähe.

Eestis on looduslikest puistutest kuusikute produktsiooni uuritud väga ulatuslikult, seda nii maapealses kui ka maa-aluses osas (Frey, 1977; Kõlli ja Kährik, 1970; Lõhmus ja Oja, 1983, 1991; Palumets, 1988, 1990, 1991, 1995), ent varemkasutatud metoodika (Orlov, 1957) ei võimaldanud alla kahemillimeetriste peente juurte produktsiooni õigesti hinnata. Ka hall-lepikute produktsiooni ja aineringet on suhteliselt hästi uuritud, juurte produktsiooni on seni hinnatud kaudselt (Lõhmus *et al.*, 1996; Mander *et al.*, 1997; Tullus *et al.*, 1996; Uri *et al.*, 2002, 2003).

Käesolevas uurimistöös on esimest korda võetud kokku uurimistulemused Eestis kasvukohatingimustest sõltuvalt alates ektomükoriisse imijuure kudede tasandist kuni kuusiku peente juurte biomassi ja produktsiooni dünaamika ning allokatsioonini. Optimaalseteks loetakse kasvukohatingimusi, mis võimaldavad maksimaalset produktsiooni. Töös on hinnatud kasvukoha mõju puude mineraaltoitumises funktsionaalselt kõige olulisematele organitele: imijuurtele, ning analüüsitud juureparameetrite dünaamikat kasvukoha kvaliteedist lähtu-

valt. Imijuurte ehituse kooskõla mullatingimustega iseloomustati anatoomiliste ja morfoloogiliste karakteristikute abil. Peente juurte biomassi, produktsiooni ja eluea määramiseks kasutati kaht meetodit: järjestikuste monoliidiseeriate ja sissekasvusilindrite meetodit, ning võrreldi tulemusi nii meetodite objektiivsust kui ka töömahukust arvestades.

Käesoleva töö peamised eesmärgid:

1) hinnata peente juurte (<1 mm ja 1–2 mm) netoproduktsiooni kõrgeboniteedilises kuusikus erinevate meetoditega (järjestikuste monoliidiseeriate ja sissekasvusilindrite meetodiga);
2) võrrelda kasutatud meetodeid nii juureproduktsiooni hinnangu objektiivsuse kui töömahukuse seisukohast;
3) hinnata peente juurte osatähtsust kuusiku netoproduktsioonis;
4) hinnata peente juurte lagunemist erinevates kasvukohtades;
5) hinnata hariliku kuuse imijuurte anatoomiliste ja morfoloogiliste parameetrite varieeruvust erinevates kasvukohtades ja analüüsida nende sõltuvust mullatingimustest;
6) analüüsida imijuurte parameetrite dünaamikat kasvukoha headusest lähtuvalt;
7) siduda ning üldistada kõik organisatoorsed tasandid (imijuur-puistu) kasvukohatingimuste optimumilähedusega.

Töö üheks olulisemaks tulemuseks on peente juurte (<2 mm) produktsiooni (2.5 t ha^{-1} a^{-1}) ja nende osatähtsuse määramine kõrgeboniteedilise kuusiku (Roela) netoproduktsioonis, mille kohta Eestis siiani puudusid objektiivsed andmed. Roela kõrgproduktiivses jänesekapsa-kuusikus läheb aastasest netoproduktsioonist maa alla 31 protsenti, millest ligi 13 protsenti moodustavad peened juured. Kirjanduse andmetega võrreldes on nimetatud juureproduktsiooni osatähtsus puistu netoproduktsioonist üks väiksem. Saadud tulemus on kooskõlas tendentsidega, et paremates kasvukohatingimustes väheneb allokatsioon juurtesse.

Järjestikuste monoliidiseeriate ja sissekasvusilindrite meetodiga hinnati võrdlevalt peente juurte erinevate diameetriklasside käibekiirust ja eluiga, mis peenema (<1 mm) fraktsiooni puhul oli monoliitides ja kolmanda aasta sissekasvusilindrites väga sarnane (0.50–0.51 aastat). Saadud tulemus jääb kirjanduses avaldatud, peamiselt minirisotronide meetodiga määratud peente juurte eluea piiridesse.

Peente juurte (<2 mm) detailsem jaotus (antud töös <1 mm ja 1–2 mm) võimaldab paremini tunnetada peente juurte produktsiooniprotsessi seaduspärasusi. Alla ühemillimeetrise jämedusklassiga fraktsioon sisaldab ektomükoriisseid imijuuri, imikasvujuuri ja peenemaid kasvujuuri, aga ka juba teiskasvule üle läinud peenikesi juhtejuuri, mille korteks on juurevarisena eraldunud. Nimetatud juurte funktsioonid on erinevad: kui imijuurte ülesandeks on tagada vee ja mineraaltoitainete omastamine, siis kasvujuurte ülesanne on hõlvata uut mullaruumi, mis võimaldab juurestikul laieneda. Peenikeste, teiskasvuga juhte-

juurte peamine ülesanne on tagada taimesisene kahesuunaline toitainete transport. Alla ühemillimeetrise läbimõõduga juured moodustavad alla kahemillimeetriste peente juurte biomassist enamuse (66%) ning peente juurte aastasest netoproduktsioonist on alla ühemillimeetriste juurte osatähtsus suurem (73%).

Peened juured diameetriga 1–2 millimeetrit on homogeensem rühm, kuhu kuuluvad enamasti kõik puitunud juhtejuured ning väike osa primaarstruktuuriga kasvujuuri, mis hiljem lähevad samuti üle teiskasvule. Viimased vastutavad juurestiku laiendamise eest mullaruumis, mis ilmnes selgesti sissekasvusilindrite katses, kus teisel aastal pärast paigaldamist oli 1–2 millimeetrise läbimõõduga juurte osakaal 49 protsenti alla kahemillimeetristest juurtest. Nende osatähtsus vähenes kolmanda aasta lõpuks 38 protsendile, mis on sarnane juba järjestikuste monoliidiseeriate tulemusega (34%), viimane peegeldab looduslikku olukorda Roela kuusikus.

Keskmiste mitmene võrdlemine näitas, et peente juurte biomass on vegetatsiooniperioodil stabiilne näitaja, biomass langeb usaldusväärselt vegetatsiooniperioodi lõppedes, novembris. Elusate juurte osatähtsuse dünaamikat kogumassist vegetatsiooniperioodi jooksul analüüsides selgus, et suurim on elusate juurte osakaal nii alla ühemillimeetriste kui ka 1–2 millimeetriste juurte fraktsioonis augustis, 1–2 millimeetriste juurte fraktsiooni puhul veel ka 1996. ja 1997. aasta juunis. Kõige väiksem erinevus aastasest keskmisest biomassist on täheldatav meie kliimatingimustes septembris-oktoobris, kui mullatemperatuur on veel plusskraadides.

Hariliku kuuse imijuured olid kõik ektomükoriisad, analüüsitud on seitsmest kuusikust kokku ligi 3500 juuretippu. Ektomükoriisad moodustasid alla ühemillimeetriste juurte fraktsioonist keskmiselt 39 protsenti. Nende olulisima funktsiooni — vee ja mineraalainete omastamise kirjeldamiseks on antud töös esitatud nii imijuurte suuruse absoluutsed kui ka funktsionaalsust iseloomustavate tuletatud ja suhteliste parameetrite varieerumispiirid erineva boniteediga kasvukohtades. Seejuures selgus, et hariliku kuuse ektomükoriisa taim- ja seenkomponent käituvad autonoomselt, kusjuures kasvukoha headust kajastavad eelkõige taimekudede dimensioonid. Hariliku kuuse ektomükoriisade anatoomia kirjeldamisel osutus otstarbekaks kasutada suhtelisi näitajaid. Ektomükoriisse imijuure korteksi ja steeli suhe oli 4:1 kasvukohatingimustest sõltumata, mistõttu teda võib käsitleda sellele puuliigile omase suurusena. Korteksi rakuridade arv eri kuusikutest kogutud proovides ei erinenud, jäädes vahemikku 4–6, ent korteksi paksus ning steeli ja juure läbimõõt oli kõrgema boniteediga kuusikutes suurem. Seega suurenes kasvukohatingimuste paranedes rakkude suurus, mitte rakuridade arv.

Ektomükoriissete imijuurte funktsionaalset efektiivsust iseloomustasid kõige paremini nende endodermi eripind, juure eripind ja eripikkus ning kudede tihedus, millest kolm esimest suurenesid ja kudede tihedus vähenes kuusiku boniteedi paranemise suunas. Uuritud kuusikute seas kõrgeima boniteediga (I^a) Roela jänesekapsa-kuusikus oli juurte tihedus väikseim (0.31 mg mm^3) ning

endodermi eripind suurim (20.8 mm^2 mg^{-1}). Füsioloogilisest aspektist talitlevad efektiivsemalt väiksema tihedusega ning suure absorbeeriva pinnaga imijuured.

Alates imijuure tasandist kuni puistu tasandini on oluline kasutada nii absoluutseid kui ka suhtelisi näitajaid, eriti suhtelised parameetrid võimaldavad tulemusi võrrelda kirjanduses avaldatud teistsuguste kliima- ja kasvukohatingimustega töödega ja seeläbi saab paremini väljendada metsaökosüsteemi funktsionaalset optimaalsust.

ACKNOWLEDGEMENTS

I wish to express my special gratitude to my supervisor, Krista Lõhmus, for introducing me to the "hidden half" of the forest, sharing with me the secrets of the "world" below the ground, for taking time with me and for appreciating me as young collegue since I was a 15-year-old schoolgirl. Your scientific questions pushed me to read more and more. Thanks also for all those morning coffees....you really were my "academic mother" through all these years.

I would like to express my gratitude to my supervisor, Olevi Kull, for comprehensive answers to my questions about the functioning of the above-ground parts of trees and for useful suggestions to manuscript of this work.

I am grateful to my assistants in laboratory works, Marju Adams and Kristiina Lotamõis, without you, I would still be counting those over 200 000 root tips hiding in 51 grams.

My special thanks go to Katrin Pajuste for all those "muddy" sunny days at Voore research station washing soil samples and those nighttime talks with the sound of crickets and corncrakes in the background.

Many thanks to Jaak Truu for valuable help with the CANOCO calculations,

- to Jane Frey for long and interesting discussions in Lossi street,
- to Arne Sellin valuable discussions about functioning of spruce forest and for useful suggestions to manuscript of this work,
- to Mari Ivask for showing me that there are many more living things in the soil than just roots,
- to Hans Persson from the Swedish University of Agricultural Sciences for valuable discussions, excellent working facilities and support,
- to Kerstin Ahlström from SUAS for kindly introducing her methodology and for thorough technical advice for working with microscope AXIOPHOT,
- to Katrin Raud for linguistic advice in Estonian,
- to Andres Tennus from Multimedia Center in the University of Tartu for help with root photos,
- to Mai Olesk from the Laboratory of Biochemistry of the Estonian Agricultural University for making chemical analyses and for friendly collaboration.

I would like to express my gratitude to many friendly root specialists in the Vantaa Forest Research Centre of the Finnish Forest Research Institute for teaching me different research methods. I am grateful to the people of Department of Ecology and Environmental Research in Swedish University of Agricultural Sciences for support during my stay in Uppsala.

I would also like to thank all the people from the Institute of Botany and Ecology and certainly from the Institute of Geography who have supported me in different ways during these years.

I appreciate the support of colleagues and friends from Estonian Forest Conservation Area Network. I enjoyed your enthusiasm and I know now how whisper Estonian old-growth forests.

I would like to thank Mr. Ilmar Part for excellent linguistic help with original papers as well as with this thesis.

My final words of thanks, in Estonian, go to my family:
- aitäh, Ema, Sinu toetuse ja julgustuse eest, mistahes valikute ees ma ka ei seisnud;
- aitäh, Mait ja Mikk, teie mõistmise ja tunnustava toetuse eest minu teaduslikel "rännakutel" ja mis veel olulisem, et minu elus pole ainult teadus...

This study was supported by Estonian Science Foundation grants No. 671, No. 2487, No. 2627, No. 3977, No. 4895, SNS project No. 82 and by the Swedish Institute.

PUBLICATION

I

Lõhmus K, Ivask M, Ostonen I. 1995.
Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils.
In: The Finnish Forest Research Institute. Research Papers 537.
Eds. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83–87.

Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils

Krista Lõhmus[1], Mari Ivask[2] and Ivika Ostonen[1]

Introduction

The most interesting results of root investigations during the last decade are connected with fine-root productivity and turnover. These indicate that the production and replacement of fine roots in boreal forests can form a major part of net primary production (Persson 1983). Therefore, root decomposition is a key process in nutrient, mass and energy dynamics of a coniferous forest. The paper represents a summary of the results of long-term decomposition studies of finest and fine roots in Norway spruce and fine roots in Scots pine, which were carried out with the aim:

(1) to describe the dynamics of the organic matter, ash, nitrogen and energy content during decomposition;
(2) to analyze variability between different sites and incubation depths;
(3) to analyze variability between different conifer species (Norway spruce and Scots pine).

Proceeding from this, the root litter-bag technique was employed.

[1] Institute of Geography, Dept. of Nature Geography and Landscape Ecology, Tartu University, Ülikooli 18, EE-2400 Tartu, Estonia.

[2] Institute of Environmental Protection, Estonian Agricultural University, Riia 12, EE-2400 Tartu, Estonia.

Table 1. Characteristics of the permanent plots in Estonia.

A. Root decomposition and stand characteristics

Site	Canopy composition	Age (years)	Mean height (m)	Site quality class	Basal area (m^2)	Remaining amount of roots after five years (% from the initial weight)
Voore 1	10S	40	19	I^a	48.9	Spruce < 1 mm, 60 %
Voore 2	9S 1B	50	20	I	33.0	Spruce < 2 mm, 54 %
Haanja	9S 1B	45	20	I	43.2	Spruce < 2 mm, 54 %
Väätsa	9S 1B	63	25	I	29.7	—
Vigala	6S 3P	43	18	I	35.6	Spruce < 2 mm, 61 %
Putkaste	9S 1B	64	19	II	34.0	Spruce < 2 mm, 49 %
Kuusnõmme	5S 5P	73	11	IV - V	11.8	Spruce < 2 mm, 73 %
Tipu	8S 1P 1B	56	22	I	44.3	Spruce < 2 mm, 53 %
Pikasilla	7P 3S	63	11	III	26.8	Spruce < 2 mm, 70 % Pine < 2 mm, 73 %
Nõva	10P	143	15	V	10.7	Pine < 2 mm, 69 %

S - *Picea abies*, P - *Pinus sylvestris*, B - *Betula pubescens*

B. Soil characteristics

Site	Soil type	Parent material	Humus form	pH (H_2O) in 0 - 20 cm
Voore 1	Umbric Luvisol	Red-brown till	mull	5.5 - 6.0
Voore 2	Umbric Luvisol	Red-brown till	mull	4.4 - 5.6
Haanja	Dystric Podzoluvisol	Red-brown till	moder	4.3 - 5.6
Väätsa	Umbric Cambisol	Yellow grey till	mull	6.5 - 7.2
Vigala	Dystric Gleysol	Vawed clay	moder-mull	4.4 - 4.9
Putkaste	Gleyic Podzol	Aqueous sand	mor	5.3 - 5.8
Kuusnõmme	Rendzic Leptosol on pebble	Pebble till	mull	7.0 - 8.3
Tipu	Haplo-Gleyic Podzol	Fluvioglacial sand	mor	4.5 - 5.0
Pikasilla	Sombri-Ferric Podzol	Fluvioglacial sand	mor-moder	4.4 - 5.3
Nõva	Sombri-Ferric-Gleyic Podzol	Fluvioglacial sand	mor	5.7 - 6.0

Materials and methods

For Norway spruce the study of the decomposition of the finest (<1 mm in diameter) roots was carried out in a 40-year-old high site quality (I^a) Norway spruce (*Picea abies* (L.) Karst.) stand described in Ivask et al. (1991), and Lõhmus and Ivask (1994) and in Table 1 (Site Voore 1). Fine root (<2 mm in diameter) decomposition studies were conducted on 8 permanent plots in Estonia.

Stand and soil characteristics are given in Table 1 (Sites: Voore 2, Haanja, Väätsa, Vigala, Putkaste, Kuusnõmme, Tipu, Pikasilla). Scots pine decomposition of fine roots (<2 mm in diameter) was studied in Pikasilla and Nõva (Table 1).

The initial material for decomposition was collected for the Norway spruce at high-quality spruce stands: finest roots (<1 mm in diameter) at site Voore 1 and fine roots (<2 mm in diameter) at site Haanja (Table 1); for the Scots pine the fine roots were collected at the low quality pine stand Nõva (Table 1). The methods are described in Lõhmus and Ivask (1994). The mesh size of the root-litter bags was about 0.1 mm, the size of litter bags was 5x5 cm^2 for finest and 8x8 cm^2 for fine roots. Each bag contained 1000 mg of finest or about 500 mg of fine roots. One hundred bags of finest roots were incubated randomly under the forest floor and in subsequent 10 cm soil layers down to the depth of 40 cm in site Voore 1 in July 1986. The litterbags of fine roots were incubated in soil at a depth of 10 cm in July 1989. The bags were collected once or twice a year except for Voore 1 and Voore 2 sites, where the seasonal dynamics was investigated. In all initial and decomposing samples oven-dry weight, ash and energy content (by the macrocalorimeter KL-5) and nitrogen concentration (by the Kjeldahl method) was determined.

Results

1. Finest (<1 mm in diameter) spruce root decomposition

The finest spruce root decomposition dynamics was studied in site Voore 1 (Table 1) and discussed in Lõhmus and Ivask (1994). The initial N, ash and lignin concentrations were 1.29 %, 5.7 % and 34.8 % respectively, caloricity was 18.48 kJ/g. By the multiple comparison of means no significant differences were found between various depths of decomposing samples for the remaining oven-dry and ash-free mass, caloricity and N concentration. The ash-free dry weight of the samples decreased by 14.3 % of the initial dry weight during the first month; the low caloricity of the dry weight loss indicates that the main loss was formed by soluble carbohydrates with a caloricity of 17.0 (Morowitz 1968). After five years the finest roots had lost 40 % of their initial weight, half of it during the first year. Following in time the absolute amount of N in remaining material, the phases of leaching, accumulation and final release (Berg and Staaf 1981) are

observed, the mean nitrogen concentrations varied during the incubation from 1.47 to 1.78 %.

2. Fine (<2 mm in diameter) spruce root decomposition studies

The initial N, ash and lignin concentrations were 0.73 %, 1.8 % and 37.0 % respectively, the caloricity was 20.0 kJ/g. The rate of fine-root decomposition in the Voore forest (Site Voore 2 in Table 1.) is somewhat different from that of the finest roots (Lõhmus and Ivask 1994). During the first month ash-free dry weight decreased by 7.3 % of the initial dry weight, which shows that the amounts of soluble compounds in fine roots are smaller than in the finest root fraction. Due to the lower initial N concentration, 0.73 % in fine roots (for finest roots 1.29 %), the change of the absolute amount of nitrogen over time is different. An accumulation phase can be distinguished, after which a release phase (Berg and Staaf 1981) begins. During the first three years the decay rates of the finest and fine spruce roots in the same soil were similar. In different soils after the first year fine spruce roots had lost 21.0 to 32.7 % of their initial dry weight, after two years the loss was 22.5 to 43.2 %. The remaining dry weight percentages from the initial dry weight after five years are given in Table 1. In all sites the N concentration in five years was higher than the initial concentration and varied from 0.97 to 1.70 % in different sites.

3. Fine (<2 mm in diameter) pine root decomposition studies

The initial N, ash and lignin concentrations were 0.47 %, 1.1 % and 22.7 % respectively, the caloricity was 19.2 kJ/g. After the first year fine pine roots had lost of their initial dry weight at Pikasilla and Nõva sites 19 % and 31 %, respectively; the nitrogen concentration varied in five years from 0.62 to 1.12 %. The remaining dry weight percentages after five years are given in Table 1.

We may conclude that the decay rates of the Norway spruce and Scots pine fine root litter are smaller at the sites where the site quality is lower.

References

Berg, B. & Staaf, H. 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. In: Terrestrial Nitrogen Cycles. Clark, F. E. & Rosswall T. (eds.). Ecol. Bull., Stockholm, 33 pp. 163-178.

Ivask, M., Lõhmus, K. & Rästa, E. 1991. Below-ground tree productivity of Norway spruce forest: a preliminary report. In: Plant Roots & their Environment. Developments in Agricultural & Managed-Forest Ecology 24. McMichael, B.L. & Persson, H. (eds.) Elsevier, The Netherlands. pp. 213-217.

Lohmus, K. & Ivask, M. 1994. Decomposition and nitrogen dynamics of fine roots of Norway spruce (*Picea abies* (L.) Karst. at different sites. Plant and Soil (in press).

Morowitz, H. J. 1968. Energy flow in biology. Acad. Press, New York, pp. 1-179.

Persson, H. 1983. The distribution and productivity of fine roots in boreal forests. Plant and Soil 71: 87-101.

II

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

Plant and Soil **208**: 283–292, 1999.

The role of soil conditions in fine root ecomorphology in Norway spruce (*Picea abies* (L.) Karst.)

Ivika Ostonen[1], Krista Lõhmus[2] and Rein Lasn[3]
[1]*Institute of Botany and Ecology, University of Tartu, Lai 40, 51005 Tartu, Estonia** [2]*Institute of Geography, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia and* [3]*Institute of Zoology and Botany, Estonian Agricultural University, Riia 181, 51014 Tartu, Estonia*

Received 15 May 1998. Accepted in revised form 27 January 1999

Key words: morphological variability, *Picea abies*, root density, specific root area, short roots, soil conditions

Abstract

The present study is an attempt to investigate the pattern of morphological variability of the short roots of Norway spruce (*Picea abies* (L.) Karst.) growing in different soils. Five root parameters – diameter, length and dry weight of the root tip, root density (dry weight per water-saturated volume) and specific root area (absorbing area of dry weight unit) were studied with respect to 11 soil characteristics using CANOCO RDA analysis. The investigation was conducted in seven study areas in Estonia differing in site quality class and soil type. Ten root samples per study area were collected randomly from the forest floor and from the 20 cm soil surface layer. Eleven soil parameters were included in the study: humus content, specific soil surface area, field capacity, soil bulk density, pH (KCl and H_2O dilution's), N and Ca concentrations, Ca/Al and C/N ratios, and the decomposition rate of fine roots (<2 mm dia.). Root morphological characteristics most strongly related to the measured soil characteristics in the different sites were specific root area, root density and diameter of the short roots, the means varying from 29 to 42 m^2 kg^{-1}, from 310 to 540 kg m^{-3} and from 0.26 to 0.32 mm, respectively; root density being most sensitive. The most favourable site and soil types resulting in fine roots with morphological characteristics for optimizing nutrient uptake (e.g. low short root density and high specific root area) were Umbric Luvisol (*Oxalis*), Dystric Gleysol (*Oxalis*) and Gleyic Luvisol (*Hepatica*). These soil types correspond to highly productive natural forest stands of Norway spruce in Estonia. All measured soil variables explained 28% of total variance of the root characteristics. The most important variables related to root morphology were the humus content, field capacity and specific soil surface area.

Introduction

The short roots of a spruce are mycorrhizal roots with primary structure and are functionally adapted to the uptake of water and mineral nutrients. The mycorrhizal short roots are morphologically distinct from the rest of the root system (Vogt and Persson, 1991). They are more variable morphologically due to symbiosis with different fungal species and their short life-span (Lõhmus et al., 1989). Root morphology is genetically controlled but also influenced by environmental factors. A large number of biotic and abiotic factors, including soil moisture and nutrients as well as fungi, bacteria and other soil organisms influence the formation of the morphological characteristics of fine roots (Kramer and Kozlowski, 1991). Short roots in the soil vary greatly with respect to morphological features. By quantifying these features, it is possible to determine morphological indices of the fine root system which also express functional aspects of fine roots. Such indices include specific root area (Orlov, 1955; Lõhmus et al., 1989, 1991), specific root length

* FAX No: +372-7-441-272. E-mail: ivika@ut.ee

Table 1. Characteristics of spruce stands (Lõhmus and Ivask, 1995)

Area	Forest site type	Basal area (m^2 ha^{-1})	Age (years)	Site quality (class)
Väätsa	*Aegopodium* spruce forest	29.7	63	I
Kuusnõmme	*Calamagrostis* spruce forest	11.8	73	IV–V
Roela	*Oxalis* spruce forest	48.9	40	I[a]
Vigala	*Oxalis* spruce forest (drained)	35.6	43	I
Putkaste	*Hepatica* spruce forest (drained)	34.0	64	II
Pikasilla	*Vaccinum vitis-idea* pine forest	26.8	63	III
Tipu	*Vaccinium myrtillus* spruce forest (drained)	44.3	56	I

(Fitter, 1985; Clemensson-Lindell, 1994; Persson et al., 1998), mean diameter and root dry weight and length (Ford and Deans, 1977).

Maximum productivity is usually considered indicative of environmental optimality. However, in determining optimal growth of short roots, morphological as well as functional parameters should be considered in relation to environmental conditions. In optimal site conditions, both the root/shoot ratio and the proportion of roots in net production may decrease (Vogt et al., 1987; Olsthoorn, 1991). It has been suggested that plants adapt to changes in nutrient availability by differentiating their morphological features (Robinson and Rorison, 1983). Morphological characteristics have been found to vary both with soil nutrient characteristics and physical-chemical conditions (Fitter, 1985; Lõhmus et al., 1989, 1991). Changes in root morphology affect ion uptake, especially when ion mobility is low (Nye, 1973; Robinson and Rorison, 1983). There has been little research on the effect of different soil types on the morphological features of mycorrhizal short roots.

In this study the ecomorphology of short roots was characterized by investigating the following parameters: diameter, dry weight and length of root tips; specific root area characterizing absorbing area of a mass unit and root density characterizing integrally their internal structure. The aim of the study was to evaluate variability of the ecomorphological parameters of short roots at different sites and to analyze their dependence on soil conditions.

Material and methods

Site descriptions

The study was carried out on seven permanent Norway spruce (*Picea abies* (L.) Karst.) stands in Estonia with each stand representing different forest types and site quality classes; stand characteristics have been described by Lõhmus and Ivask (1995) and are presented in Table 1.

Sampling and processing of roots

Root samples were collected from seven permanent plots of 50 × 50 m established in 1987 in middle-aged coniferous stands and located throughout Estonia. From each study plot 10 root samples were randomly collected from the forest floor and from the 20 cm soil surface layer using a spade in October 1995. In Roela the sampling was carried out in April 1995. The excavated root fragments were not separated from different soil horizons. Roots were washed with tap water to remove mineral soil. Three random subsamples were taken from each sample. The number of short root tips from each set of 30 subsamples totalled 309–464. The roots were cleaned to remove all soil particles using a small soft brush and finally washed with distilled water. The root tips were examined under a binocular microscope, counted and photographed in order to measure their projection area and diameter. In order to ensure comparability of morphological measurements the roots were water saturated when photographed. The roots were dried at 105 °C for 2 h to constant weight and weighed. The photographs (magnification 10×) of short roots were scanned and digitized. The area of the digitized images was measured using a program PINDALA, version 1.0 (designed by I. Kalamees, Eesti Loodusfoto, Tartu, Estonia). All projected area were calibrated separately using the standard of known area. To obtain the mean diameter, the diameters (d_j) of all short root branches on photos were measured with a 1 mm step (10 mm on photos).

Root characteristics

Five parameters of short roots characterizing both their morphological and functional features were determined: mean diameter, mean length and dry weight of root tip, root density and specific root area.

Geometrically, the short roots of a Norway spruce can be considered as cylinders with varying diameter

and length. Although the apexes of short roots are round, deviation from the shape of a cylinder can be considered insignificant, because the mean error caused by roundness of tips does not exceed 2% (Lõhmus et al., 1989). Hence, the total surface area of absorbing roots (S) is

$$S = \pi \sum_{i=1}^{n} d_i l_i \quad (1)$$

and the volume (V) is

$$V = 0.25\pi \sum_{i=1}^{n} d_i^2 l_i, \quad (2)$$

where l = root length, d = root diameter and n = the number of short root tips. The mean diameter (D) was calculated as

$$D = \frac{1}{k} \sum_{j=1}^{k} d_j, \quad (3)$$

where k = the number of diameter measurements in a sample.

The mean root tips length was calculated as

$$length = \frac{S}{n\pi D}, \quad (4)$$

where S = the surface area (1), D = mean diameter (3) and n = number of short root tips.

The mean dry weight of root tips, root density (RD) and specific root area (SRA) were calculated as follows:

$$weight = \frac{M}{n}, \quad (5)$$

$$RD = \frac{M}{V}, \quad (6)$$

$$SRA = \frac{S}{M}, \quad (7)$$

where M = the dry weight of the sample, n = number of root tips, V = the volume of short roots in the sample (2) and S = the surface area (1).

Specific root area, a characteristic of root–soil contact, is inversely proportional to diameter and root density:

$$SRA = \frac{S}{M} = \frac{\pi \cdot D \cdot L}{RD \cdot V} = \frac{\pi \cdot D\dot{L}}{RD \cdot \frac{\pi \cdot D^2}{4} \cdot L} = \frac{4}{RD \cdot D} \quad (8)$$

Specific root length, SRL (the length-to-dry-weight) was calculated as follows:

$$SRL = \frac{L}{M} = \frac{4}{\pi \cdot RD \cdot D^2}. \quad (9)$$

Specific root length was not included in the RDA analysis, but values were compared to literature data.

Soil characteristics

Ca concentration was determined photometrically and Al (with aluminum), spectrocolorimetrically from soil samples (Lõhmus and Lasn, 1990). Total N was determined by the Kjeldahl method. The specific surface area of the soil (S) was determined by the Puri and Murari method (1964). Methods for the determination of humus content, bulk soil density and the decomposition rate of fine roots have been described in Lõhmus et al. (1989, 1995) and for field capacity in Brady (1990).

Statistics

Principal component analysis (PCA) and redundancy analysis (RDA) (CANOCO programme: Ter Braak, 1987) were used to detect relationships between root characteristics and soil parameters. The significance of RDA analysis results was tested with a permutation test ($p < 0.01$). Root parameters of different stands were compared using multiple comparison of means (Tukey test; $p < 0.05$). Variables were checked for normality and variance homogeneity.

Results and discussion

Root variability

Table 1 provides the site characteristics of each Norway spruce stand and Table 2 highlights the physical and chemical features of the soil at each site. Mean characteristics of Norway spruce short roots on permanent plots are presented in Table 3. The results of principal component analysis for root characteristics show that two axes account for 72% of the total variation of morphological parameters of absorbing roots, while three axes account for 99%. Root density and specific root area were correlated with the first axis, mean root tip length and dry weight with the second axis, and the mean diameter with the third axis. The soil variables explained the largest proportion of the variation associated with fine root morphological state (short root density and surface area).

The density of short roots varied between different parts of the root system and among the root tips. Young, unsuberized, parts of growing roots were relatively low in dry matter; root tissue density increases as

Table 2. Physical and chemical characteristics of the soil in the investigation

Area	Humus form	Soil type	H (%)	S ($m^2\ g^{-1}$)	Field capacity (mm)	D_m ($g\ cm^{-3}$)	pH–KCl	pH–H_2O	N concentration ($mg\ 100g^{-1}$)	Ca/Al	C/N	Ca ($mg\ 100\ g^{-1}$)	Root decomposition (expressed as % of initial weight)
Väätsa	mull	Umbric Cambisol	6.21	66.74	35.27	1.1	6.1	6.8	0.13	0.40	27.70	82.5	–
Kuusnõmme	mull	Rendzic Leptosol	3.71	97.95	34.14	0.9	7.9	7.7	0.18	7.10	11.63	1170	73
Roela	mull	Umbric Luvisol	4.05	57.23	33.89	1.1	4.6	5.75	0.14	0.07	16.78	20.6	54
Vigala	moder-mull	Dystric Gleysol	9.3	79.78	32.72	0.9	3.6	4.75	0.14	0.30	38.45	33.8	61
Putkaste	moder-mor	Gleyic Podzol	8.5	69.77	30.34	1.1	4.5	5.7	0.17	1.16	28.97	175.3	49
Pikasilla	mor	Sombri-Ferric Podzol	2.65	30.20	28.86	1.25	4.3	5.0	0.06	0.10	25.62	25.3	70
Tipu	moder	Haplo-Gleyic Podzol	4.0	32.07	29.70	0.85	3.9	5.2	0.18	0.60	12.89	27.9	53

H – humus content; S – specific soil surface area; D_m – bulk density.

the fine root become suberized. Since suberin in cortex functions as a permeability barrier blocking apoplastic transport of substances (Clarkson and Robards, 1975; Peterson, 1988), density can be considered a measure of root functional status for Norway spruce. Since most of the short roots of Norway spruce are ectomycorrhizal, both their dry weight and volume depend on how they respond to fungal symbiont species found colonizing the root tips and soil characteristics. In addition specific root area has been recorded to vary from 28 to 63 m^2 per kg dry weight in Norway spruce (Abrazhko, 1973, 1985; Lõhmus and Oja, 1983; Lõhmus et al., 1989), in that case the means of different sites varied from 29 to 42 $m^2\ kg^{-1}$. Changes in specific root area are probably a way for plants to respond to changes in environmental conditions (Lõhmus et al., 1989). Thus, root density and specific root area of short roots are connected with the physiological activity of fine roots.

In this study, mean root density varied from 310 to 540 kg m^{-3}, in other words 1.7-fold in the investigated stands and was higher in spruce growing in low quality sites; the range varied from 180 to 680 kg m^{-3}. Total variation includes a genetically-determined component and changes caused by abiotic and biotic environmental factors. Among biotic factors, root density of a mycorrhizal short root is influenced by the fungal symbionts, because the proportion of the mantle to the root volume and biomass may vary with different ectomycorrhizal types. The number of different species of ectomycorrhizal fungi found in fine roots of Norway spruce and morphological types of ectomycorrhiza can be quite high (Söderström and Bååth, 1978; Egli et al., 1993). Fungal symbionts found on the roots of a particular species of plant will also vary with soil type. However, not all mycorrhizas reveal species specific features in their morphology and anatomy which are correlated to visual identification of ectomycorrhizal types (Brunner, 1991). As Egli et al. (1993) reported, the number of different ectomycorrhizal types that were identified using macroscopic and microscopic features was smaller than the number of ectomycorrhizal fungal species. In exceptional cases, one single fungus can be identical with one type, e.g. *Cenococcum graniforme* (Egli et al., 1993). Although classification of ectomycorrhizal types was not dealt with in this study, differences in mantle features, particularly in color and mantle surface structure (Agerer, 1987–1993) were revealed within a site and between different sites. In Roela (in August 1992) we compared root density and specific root area and the mean diameter of short roots for two groups of ectomycorrhizae, one group representing *Cenococcum* sp. and the second all other types. Mean values for *Cenococcum* sp. and for the other types were for root density 178 ± 7 and 243 ± 7 kg m^{-3}; for diameter 0.54 ± 0.02 and 0.38 ± 0.01 mm, respectively, the differences were statistically significant (Tukey test at

Table 3. Mean morphological characteristics of short roots (± mean standard errors). Significant differences between study areas (Tukey test, $p<0.05$) are shown by letters

Area	Specific root area ($m^2\ kg^{-1}$)	Root density ($kg\ m^{-3}$)	Diameter (mm)	Length (mm)	Weight (mg)
Roela	40.9 ± 1.1^a	310 ± 10^a	0.32 ± 0.01^a	1.37 ± 0.08	0.034 ± 0.002
Putkaste	42.0 ± 1.1^a	320 ± 10^a	0.31 ± 0.01^a	1.72 ± 0.15	0.039 ± 0.003
Vigala	42.4 ± 0.9^a	340 ± 20^a	0.29 ± 0.01^{ab}	1.51 ± 0.11	0.032 ± 0.002
Kuusnõmme	33.6 ± 1.2^b	400 ± 10^b	0.31 ± 0.01^a	1.41 ± 0.10	0.040 ± 0.003
Väätsa	31.5 ± 1.1^b	410 ± 20^b	0.32 ± 0.01^a	1.54 ± 0.11	0.050 ± 0.004
Tipu	34.8 ± 1.1^b	410 ± 10^b	0.29 ± 0.01^{ab}	1.78 ± 0.11	0.046 ± 0.003
Pikasilla	29.4 ± 1.0^b	540 ± 20^c	0.26 ± 0.01^b	1.71 ± 0.11	0.046 ± 0.002

$p < 0.05$). However, differences in specific root area (*C.* sp.: 41.82 ± 1.36; others: $43.15 \pm 0.94\ m^2\ kg^{-1}$) were insignificant. Thus, mycorrhizal diversity and its effect on the investigated root characteristics may be significant. The root density was lower in August than in April (Table 3). Although not included here, all investigated ecomorphological root characteristics exhibited seasonal dynamics.

Diameter accounted for less than one third of the total variability of root density. The individual root diameter varies widely within species and is determined by cell size in the stele and surrounding tissues (endodermis, cortex, epidermis) which provide transport to and from the root tip and absorbing cells (Lyford, 1975; Fitter, 1987). The diameter of short roots tended to increase with more favourable soil conditions (Figure 3) and the means varied from 0.26 to 0.32 mm in the different sites. However, because the diameter distributions between study areas varied more than their mean diameters (Figure 4; Table 3), mean diameter does not appear to be a sensitive parameter. The mean diameter of short roots in Pikasilla was significantly smaller than in Roela, Putkaste, Väätsa and Kuusnõmme; the differences between Pikasilla, Vigala and Tipu were insignificant (Figure 5).

Specific root length varied from 30.8 to 47.2 m g^{-1} which is more than three times higher than SRL for fine roots (<1 mm dia.) found by Persson et al. (1998). Fraction of fine roots is morphologically not homogeneous and includes roots with primary and secondary structure. Only short roots with primary structure have been involved in this study. Thus, there should be differences between diameter and/or root density values of short roots with primary structure and secondary woody roots, respectively.

Root–soil relationship

RDA analysis of 11 soil parameters from Table 2 and five root characteristics are displayed on a biplot (Figure 1) in which the present their relationships. RDA results of soil variables and sites are displayed on the same biplot.

Soil variables explained 28% and sites 29% of the total ecomorphological variability of short roots ($p < 0.01$). Soil humus content, a general measure of soil fertility, described most of the total variability (10%), followed by the soil C/N ratio, field capacity, pH–H_2O, specific soil surface area and pH–KCl. Other soil characteristics did not account for additional variation. Field capacity and specific soil surface area (an indirect measure of the water regime) reflected soil-water conditions best; without them the total variance explained by all soil characteristics decreased by one fifth (5.3%). Thus, soil nutrient regime followed by water regime, described by the humus content, field capacity and specific soil surface area are most important soil variables in terms of explaining the variability of characteristics of short roots in Norway spruce.

Canonical ordination is unable to detect statistically significant differences in the root parameters from different sites. However, significant differences were established by multiple comparison of means. Three groups were formed with respect to root density: (1) Roela, Putkaste, Vigala; (2) Tipu, Väätsa, Kuusnõmme and (3) Pikasilla, and two groups with respect to specific root area: (1) Roela, Putkaste, Vigala and (2) Tipu, Väätsa, Kuusnõmme, Pikasilla. It should be noted that an inverse relationship exists between root density and specific root area for the Roela, Putkaste and Vigala sites (Figure 2). In these sites the root density was significantly smaller and specific root area,

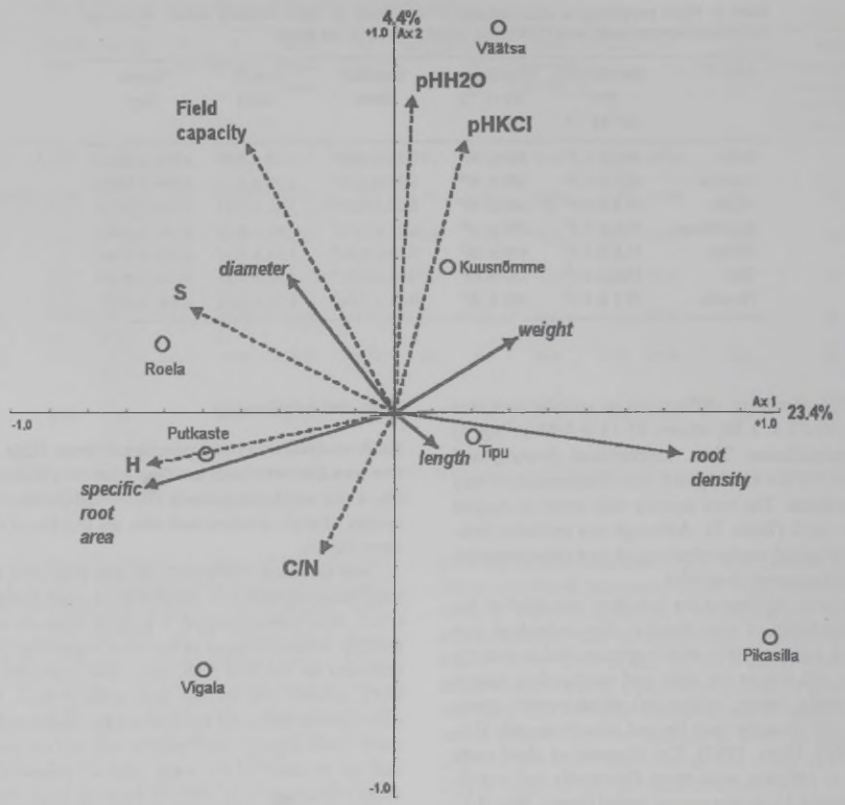

Figure 1. Biplot of RDA analysis. Solid lines are root characteristics, dashed lines soil parameters and circles are different study areas and corresponding forest sites.

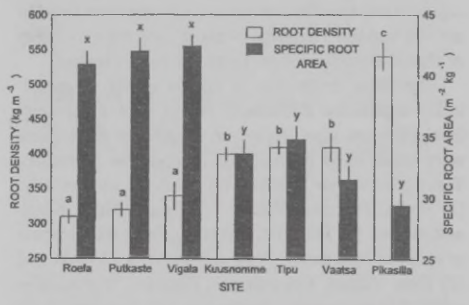

Figure 2. Mean characteristics of Norway spruce absorbing roots in 7 study areas. Bars indicate standard errors. Letters denote statistically significant differences between means; a, b, c between root density values and x, y between specific root area values.

correspondingly higher than the other stands (Figure 2). These stands are associated with optimal soil conditions, connected with the first ordination axis, and corresponding site types are positively correlated to the increasing of humus content (H), field capacity and specific soil surface area (S) (Figure 1). Root density decreases and specific root area increases from right to left along the first axis. Ion and water uptake capacity of short roots depend more on their surface area than on dry weight (Nye and Tinker, 1977). An increase in specific root area indicates a larger surface area per dry weight. Hence, the larger is specific root area, the more effective is the economy of allocation of assimilates to short roots.

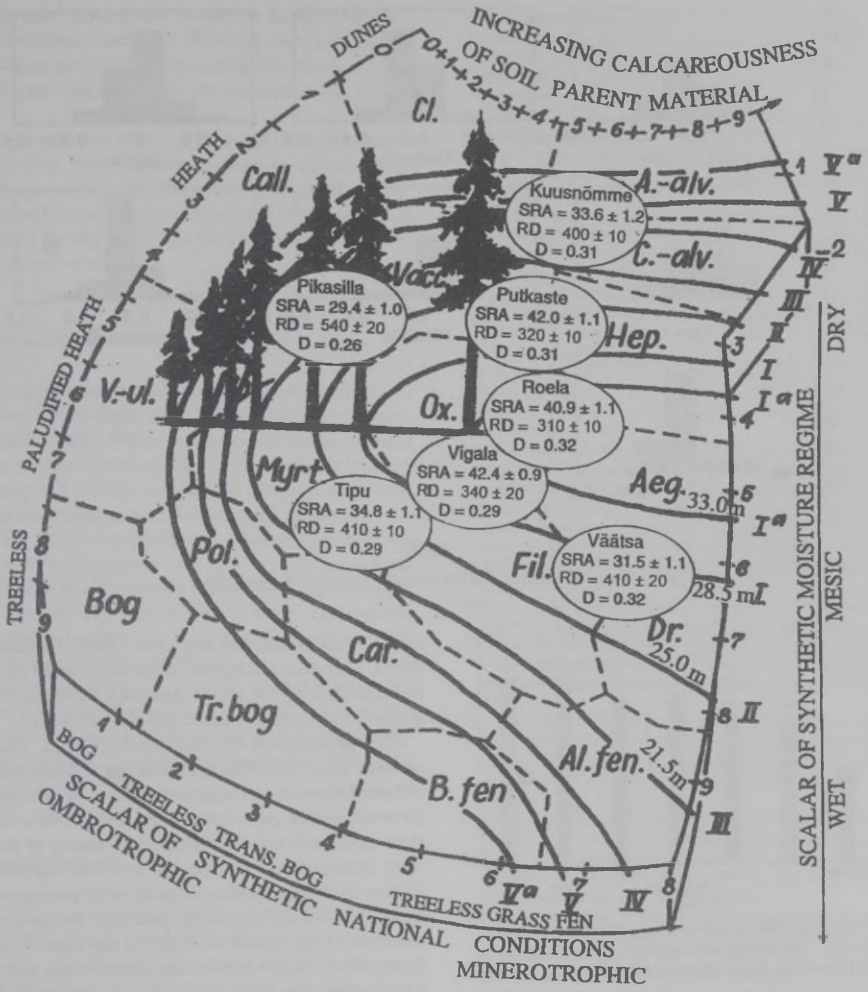

Figure 3. The average growth rate of spruce depending on the site, solid lines denote the same site class, dashed lines denote the boundary of the site type: Cl. – Cladonia; A.-alv. – *Arctostaphylos* alvar; Call. – *Calluna*; C.-alv. – *Calamagrostis* alvar; Hep. – *Hepatica*; Aeg. – *Aegopodium*; V.-ul. – *Vaccinium uliginosum*; Vacc. – *Vaccinium vitis-idea*; Ox. – *Oxalis*; Myrt. – *Myrtillus*; Fil. – *Filipendula*; Pol. – *Polytrichum*; Dr. – *Dryopteris*; Tr. bog – Transitional bog; B. fen – Birch swamp; Al. fen – Alder fen; Car. – *Carex* (Etverk et al., 1995). Study areas and root density (RD), specific root area (SRA) and diameter (D) values of short roots, respectively are located correspondingly site type and quality class. The standard error of diameter was in all study areas ± 0.01.

The natural distribution of Norway spruce in Estonia enabled us to determine the relationship between root characteristics and optimality of soil conditions. Roela *Oxalis* spruce forest is highly productive and features favourable soil conditions (Table 2). Vigala *Oxalis* spruce forest site and Putkase *Hepatica* spruce forest site are drained, which has lead to an improvement of the soil conditions. The highest productivity

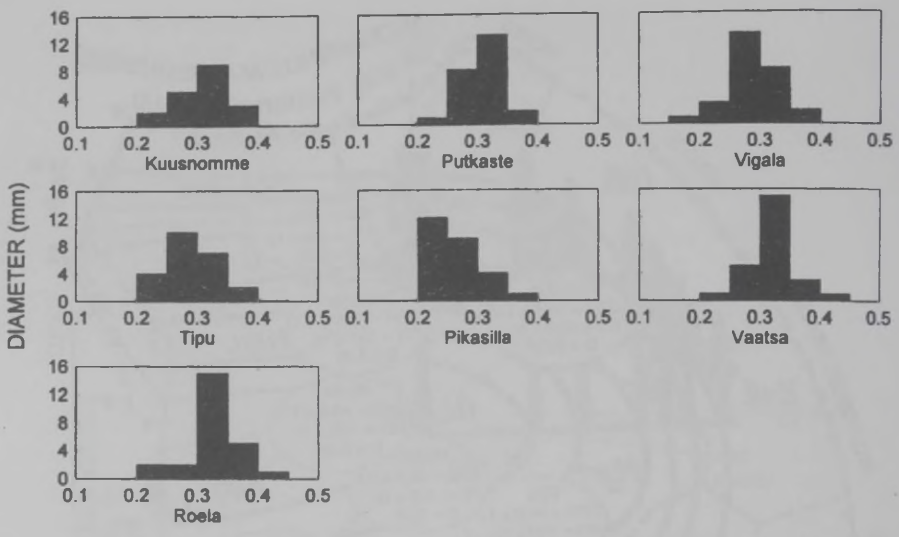

Figure 4. Frequency distribution of diameter of short roots in Norway spruce.

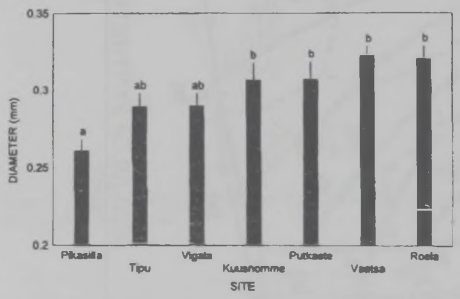

Figure 5. Mean diameter of Norway spruce short roots on investigated study plots. Bars indicate standard errors, letters a and b denote statistically significant differences after ($p < 0.005$).

of spruce is at *Aegopodium* forest site, followed by *Oxalis* and *Hepatica* forest sites (Etverk et al., 1995), where both the humus content and decomposition rate are high to medium (Lõhmus et al., 1995). It can be concluded that root density is lower and specific root area is large in spruce stands of high productivity that have grown in favourable soil conditions (Figure 3). The proportion of fine roots (including short roots) in root net primary production decreases with more optimal soil conditions (Vogt et al., 1987; Olsthoorn, 1991). This decrease can be attributed to some extent by variations in the above described morphological features and especially in decreasing root density.

The productivity of other stands (Väätsa, Kuusnõmme, Tipu, Pikasilla) is limited due to unfavorable soil conditions or root infection with a root rot fungus (*Heterobasidion annosum*). Compared to sites with more favourable soil conditions, root density of short roots increases and the specific root area decreases (Figure 2). Väätsa stand exhibits close to optimum soil conditions and site type (*Aegopodium*), but roots are deteriorated due to root rot (Lõhmus and Lasn, 1990). Comparison of root density and specific root area in *Aegopodium* site (Venevere), investigated earlier by Lõhmus et al. (1989), with the results of this study shown that these values (root density = 230 kg m^{-3} and specific root area = 43.8 m^2 kg^{-1}) were in accordance with the corresponding root characteristics of the most productive sites.

Unfavourable soil conditions at less productive sites stem from various factors. In Kuusnõmme stand (*Calamagrostis* site) spruces suffer from phosphorus deficiency due to high pH (~8) (Frey and Frey, 1995). Drainage of Tipu stand (*Vaccinium myrtillus* site) res-

ults in sand beneath the A-horizon being acidic and poor in mineral nutrients. Pikasilla stand (*Vaccinium vitis-idaea* site) is characterized by low mineral nutrient content and the lowest decomposition rate (Table 2).

A comparison of the mean length and dry weight of the short roots at different Norway spruce stands did not reveal any statistically significant differences. Root length varies with a species, and different factors affecting the dry weight of the root tip may counterbalance one another.

Conclusions

This study shows that the morphological variability of Norway spruce short root is related to soil conditions. The soil nutrient regime and, to lesser extent, the soil water regime (described by the humus content, field capacity and specific soil surface area) are especially important, explaining 28% of the total variability.

The most variable attributes of short roots in *Picea abies* were root density and specific root area. Root density can be considered a characteristic of root functional status. The root density is lower and specific root area is larger in highly productive spruce stands with favourable soil conditions.

The mean diameter of short roots tended to increase with more favourable soil conditions, but does not appear to be sensitive parameter for characterizing morphological variability of short roots in different soil types.

Acknowledgments

This study was supported by Estonian Science Foundation grants No 671 and 2487. Many thanks to Jaak Truu for valuable help with the CANOCO calculations. Hans Persson and Kerstin Ahlström are thanked for suggestions and comments on the manuscript and Robert Szava-Kovats for the linguistic revision.

References

Abrazhko M A 1973 Vsasyvayushchaya poverkhnost' kornej (Absorbing surface of roots). *In* Struktura i produktivnost' elovykh lesov yuzhnoj taigi (Structure and Productivity of Spruce Forests). Ed. V G Karpov. pp 132–133. Nauka, Leningrad.

Abrazhko M A 1985 O vliyanij azotnykh udobrenij na raspredelenie I fraktsionnyi sostav kornej *Picea abies* (Pinaceae) (The effect of nitrogen fertilizers on spatial distribution and fractional composition of *Picea abies* (Pinaceae) roots). Botanitcheskij zhurnal (Sov. Bot. J.) 70, 250–254.

Agerer R 1987–1993 Colour Atlas of Ectomycorrhizae. 1st–7th edn. Schwäbisch Gmünd: Einhorn-Verlag.

Brady N C 1990 The Nature and Properties of Soils. MacMillan Publishing Company, New York. 621 p.

Brunner I 1991 Comparative studies on ectomycorrhizae synthesized with various in vitro techniques using *Picea abies* and two *Hebeloma* species. Trees 5, 90–94.

Clarkson D T and Robards A W 1975 The endodermis, its structural development and physiological role. *In* The Development and Function of Roots. Eds. A Macfadyen and E D Ford. pp 415–436. Academic Press, New York.

Clemensson-Lindell A 1994 Norway spruce fine-root morphology and function influenced by nutrient applications in forests. Dissertation. Report 72, Swedish University of Agricultural Sciences, Uppsala.

Egli S, Amiet R, Zollinger M and Schneider B 1993 Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. Trees 7, 123–129.

Etverk I, Karoles K, Lõhmus E, Meikar T, Männi R, Nurk T, Pikk J, Randveer T, Tamm Ü, Veibri U and Örd A 1995 Estonian Forests and Forestry. Estonian Forest Department, Tallinn 1995. 128 p.

Fitter A H 1985 Functional significance of root morphology and root system architecture. *In* Ecological Interactions in Soil. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 87–106. Special publication of the British Ecological Society No. 4. Blackwell Scientific, Oxford.

Fitter A H 1987 An architectural approach to the comparative ecology of plant root systems. New Phytol. 106, 61–77.

Ford E D and Deans J D 1977 Growth of a sitka spruce plantation: spatial distributionand seasonal fluctuations of lengths, weights and carbohydrate concentrations of fine roots. Plant Soil 47, 463–485.

Frey T and Frey J 1995 Foliar chemical composition of Norway spruce with and without defoliation. *In* Nutrient uptake and cycling in forest ecosystems. Eds. L O Nilsson, R F Hüttl, U T Johansson and P Mathy. pp 123–129. Ecosystem Res. Rep. 21, Brussels.

Kramer P J, Kozlowski T T and Pallardy S g 1991 The Physiological Ecology of Woody Plants. Academic Press, San Diego. 657 p.

Lõhmus K and Ivask M 1995 Decomposition and nitrogen dynamics of fine roots of Norway spruce (*Picea abies* (L.) Karst.) at different sites. Plant Soil 168–169, 89–94.

Lõhmus K, Ivask M and Ostonen I 1995 Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils. *In* Role of Roots, Mycorrhizas and Rhizospere Microbes in Carbon Cycling in Forest Soil. Extended abstracts of the NorFa – workshop, Eds. H-S Helmisaari, A Smolander and A Suokas. pp 83–87. Research Papers 537, The Finnish Forest Research Institute, Helsinki.

Lõhmus K and Lasn R 1990 Spruce and pine root structures and chemical characteristics in moderate acid soils. *In* Above- and Below-ground Interactions in Forest Trees in Acidified Soils. Ed. H Persson. pp 74–78. Air Pollution Research Report 32, Commission of the European Communities, Uppsala.

Lõhmus K, Lasn R and Oja T 1991 The influence of climatic and soil physical conditions on growth and morphology of Norway spruce roots. *In* Plant Roots and Their Environment. Eds. B L McMichael and H Persson. pp 233–239. Elsevier Science Publishers, Amsterdam.

Lõhmus K and Oja T 1983 K metodike izucheniya podzemnoj chasti drevostoev (On methods of studying below-ground growth of a forest stand). Lesovedenie (Sov. For.) 4, 56–62.

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

Lyford W H 1975 Rhizography of non-woody roots of trees in the forest floor. *In* The Development and Function of Roots. Eds. J G Torrey and D T Clarkson. pp 179–196. Academic Press, New York.

Nye P H 1973 The relation between radius of a root and its nutrient absorbing power (α). J. Exp. Bot. 24, 783–786.

Nye P H and Tinker P B 1977 Solute movement on the soil – root system. pp 342. Blackwell, Oxford.

Olsthoorn A F M 1991 Fine root density and root biomass of two Douglas-fir stands on sandy soils in The Netherlands: 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth. J. Agric. Sci. 39(1), 61–77.

Orlov A Ya 1955 K metodike kolichestvennogo opredeleniya sosuschikh kornej drevesnykh porod v pochve (On the methods of quantitative determination of absorbing tree roots in soil). Byulleten MOIP. Otd. biol. (Bull. Moscow Nature Res. Soc. Biol. Sec.) 60, 93–103.

Persson H, Ahlström K and Clemensson-Lindell A 1998 Nitrogen addition and removal at Gårdsjön – effects on fine-root growth and fine-root chemistry. For. Ecol. Manage. 101, 199–205.

Peterson C A 1988 Exodermal Casparian bands: their significance for ion uptake by roots. Physiol. Plant. 72, 204–208.

Puri B and Murari K 1964 Studies in surface-area measurements of soils. 2, Surface area from a single point on the water isoterm. Soil Sci. 97, 341–343.

Robinson D and Rorison I H 1983 Relationships between root morphology and nitrogen availability in a recent theoretical model describing nitrogen uptake from soil. Plant Cell Environ. 6, 641–647.

Söderström B E and Bååth E 1978 Soil microfungi in three Swedish coniferous forests. Hol. Ecol. 1, 62–72.

Ter Braak C J F 1987 CANOCO – a FORTRAN Program for Canonical Community Ordination by [Partial] [Detrended] [Canonical] Correspondence Analysis. Principal Components Analysis and Redundancy Analysis (Version 2.1). 95 p. Agriculture Mathematics Group, Wageningen.

Vogt K A and Persson H 1991 Measuring growth and development of roots. *In* Techniques and Approaches in Forest Tree Ecophysiology. Eds. J P Lassoie and Th M Hinckley. pp 477–501. CRC Press, Boston.

Vogt K A, Vogt D J, Moore E E, Fatuga M B, Redlin M R and Edmonds R L 1987 Conifer and angiosperm fine-root biomass in relation to stand age site productivity in Douglas-fir forests. J. Ecol. 75, 857–870.

Section editor: H Lambers

III

Ivask M, Truu J, Truu M, Lõhmus K, Ostonen I. 1999.
The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia.
Baltic Forestry, 2: 32–36

The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia

MARI IVASK, JAAK TRUU, MARIKA TRUU
Environmental Protection Institute of the Estonian Agricultural University, Kreutzwaldi 5, 51014Tartu, Estonia
KRISTA LÕHMUS
Institute of Geography, University of Tartu, Vanemuise 46, 51014Tartu, Estonia
IVIKA OSTONEN
Institute of Botany and Ecology, University of Tartu, Lai 40, 51005Tartu, Estonia

Ivask M., Truu J., Truu M., Lõhmus K., Ostonen I. 1999. The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia. *Baltic Forestry.* 2: 32–36.

The forest floor is an important compartment in soil processes and nutrient cycling. The decomposer communities in four Norway spruce (*Picea abies* (L.) Karst) forests and in two Scots pine (*Pinus silvestris* L.) forests were studied. The number of earthworm individuals (0...88) and species (0...5) was variable in forest floor of studied coniferous forests. 0...28 earthworm individuals per 1 m² and 0...3 species was found in mineral soil of studied conifer forests and 104±7 individuals per 1 m² and 7 species, as exception, in the spruce forest with abundant grass layer. In spruce forests the total number of earthworm individuals was 15...124 per 1 m² and the total number of species was 2...8, in pine forests - 0...8 individuals per 1 m² and 0...2 species was found. The mean microbial activity in the forest floor of spruce forests was 4,636±1,04 OD/g, in pine forests 3,094±0,69 OD/g.

Key words: earthworm community, forest floor, microbial activity, Norway spruce, Scots pine.

Introduction

Litter decomposition is a key process in all terrestrial ecosystems because it controls nutrient availability and hence primary production (Rutigliano et al., 1996). Litter quality is therefore an important factor in the transfer of energy and plant nutrients to forest soils. The abundance and composition of soil organisms in forest floor and upper soil layer may also influence the rate of decomposition, and subsequently the release of mineral nutrients. In the same time, the community structure and composition of soil and litter organisms is affected by tree species (Saetre, 1998). The community of decomposers consists of micro-organisms and soil faunal organisms; soil fauna are known to be responsible for as much as 30% of the N and C mineralisation (Görres et al, 1998). Earthworms have frequently been considered as one of the most important faunal decomposers in soil because of their effects on soil structure formation and on nutrient cycling.

The aim of the present study is to describe the structure of earthworm community and the microbial activity in spruce and pine forests in Estonia, and to examine the influence of characteristics of forest floor on the parameters of earthworm communities.

Materials and methods

The structure and the relationships of the main decomposer, microbial and earthworm communities in four Norway spruce (*Picea abies* (L.) Karst) forests and in two Scots pine (*Pinus silvestris* L.) forests were studied. Characteristics of stands and soil are given in table 1, descriptions of study areas are published in Lõhmus et al. (1995). The study areas were selected according to the results of our earlier studies on long-term decomposition of fine roots (Lõhmus et al, 1995). The forest floor and soil samples for chemical and microbiological analysis were collected in May and June, on the quadrates 50 x 50 cm or on the rings ∅ 104 mm. The thickness of forest floor layer was measured. The number of sample areas was 10...25, depending on the variability of samples (the mean error of thickness of forest floor does not exceed 10%). The samples were cleaned, sorted, weighed and dried at 75°C. The oven-dry weight and moisture content in all samples, and

organic matter content (in muffel oven at 360°C), nitrogen concentration (by Kjehldal method) and soluble phosphorus concentration (by lactate method) in a composite sample were determined. Soil samples were taken on the same quadrates or the rings, in the upper soil layer 0...15 cm. In all samples moisture content, in a composite sample organic matter content (in muffel oven at 360°C), nitrogen concentration (by the Kjeldahl method) and soluble phosphorus concentration (by lactate method) were determined.

Total activity of microbial community as one of essential factors of habitat for earthworms has been measured using fluorescein diacetate method that estimates the activity of dehydrogenase enzymes in a composite sample (Schnürer and Roswall, 1985). Total activity of microorganisms measured in optical density units (OD) characterises metabolic activity of microbial community and correlates well with CO_2 evolution from soils. The total activity of soil microbes is expressed per 1 g of dry soil, but organic matter content has been also recommended for normalisation of soil microbiological parameters, because the microorganisms are associated with organic matter of substrate.

Earthworm communities are characterized by high seasonal variability in the number of individuals, they were collected in October at the time of maximum density, greatest activity and lowest variability of individuals (Nordström and Rundgren, 1973). The samples were collected from five soil blocks measuring 50 × 50 × 40 cm, by hand sorting (Satchell, 1967), separately from forest floor and soil; the earthworms were washed and identified to species.

For all sampling occasions and earthworm species mean numbers of individuals per 1 m² and their standard errors were calculated. Regression analysis was used and the correlation coefficients were established. Variables were checked for variance homogeneity.

Results and discussion

The stands are characterised by different age (43...143 years), site quality class (I...V) and humus form (mull, moder-mull, moder-mor or mor) (Table 1). Decomposition rate of fine roots was highest in Värska pine forest and lowest in Kuusnõmme spruce forest (table 1). In studied spruce forests, the forest floor layer was thickest in Vigala (88,7±4,5 mm, 11,4±0,97 kg m^{-2}) study area. On the study area Kuusnõmme the forest floor layer on the surface of soil is missing but the ground vegetation of grasses is abundant. In pine forests, the forest floor layer was the thickest in Nõva (50,8±2,7 mm, 10,3±1,4 kg m^{-2}) (Table 2). All studied forests differed by dry matter and organic matter content in forest floor (22,8...39,1% and 41,6...76,8%, respectively) and soil (49,4...96,0% and 0,7...22,2%, respectively) (Table 3). The mean pH of forest floor and soil was 5,1±0,2 and 4,7±1,0 in spruce forests, and 3,5±0,3 and 4,3±0,1 in pine forest, respectively. The mean nitrogen content of forest floor and mineral soil was 1,61±0,22% and 0,56±0,16% in spruce forests and 1,09±0,16% and 0,02±0,01% in pine forests. The mean soluble phosphorus content was very variable: from 5,0 to 22,7 mg P per 100 g of dry substrate in forest floor and from 0,4 to 40,1 mg P per 100 g dry substrate in soil of all studied forests.

The activity of microbes was higher in forest floor layer as compared to soil on all studied sites (Table 3).

Table 1. Characteristics of stand and soil on study areas (by Lõhmus et al., 1995).

Site	Canopy composition	Age (years)	Site quality class	Soil type	Humus form	Remaining amount of roots after five years (% from the initial weight)
Voore	9S1B	50	I	Umbric Luvisol	mull	54
Vigala	6S3P	43	I	Dystric Gleysol	moder-mull	61
Putkaste	9S1B	64	II	Gleyic Podzol	moder-mor	49
Kuusnõmme	5S5P	73	IV-V	Rendzic Leptosol	mull	73
Nõva	10P	143	V	Sombri-Ferric-Gleyic-Podzol	mor	69
Värska	10P	54	III	Podzol	mor	40

S - *Picea abies*, P - *Pinus silvestris*, B - *Betula pubescens*

Table 2. Characteristics of forest floor.

Site	Stand	Thickness of layer, mm	Dry weight per 1 m²
Voore	Spruce	24,2±2,5	1520±190
Vigala	Spruce	88,7±4,5	11400±970
Putkaste	Spruce	31,1±1,8	1700±220
Kuusnõmme	Spruce	–0	–0
Nõva	Pine	50,8±2,7	10300±1400
Värska	Pine	63,4±1,8	3850±170

Table 3. Activity of micro-organisms in the soil and forest floor calculated per 1 g of dry soil (Activity 1, OD/g) and per organic matter content (Activity 2, OD/OM), and ratios of microbial activities in the soil and forest floor and soil on different sites.

Site	Fraction	Activity 1 OD/g (mean±SE)	Activity 2 OD/OM (mean±SE)	Ratio1	Ratio 2
Voore	soil	0,868±0,012	8,092±0,147	3,860	0,251
Voore	forest floor	3,352±0,019	2,034±0,179		
Vigala	soil	3,008±0,244	9,763±1,593	2,227	0,257
Vigala	forest floor	6,697±0,205	2,509±0,291		
Putkaste	soil	2,384±0,157	6,222±0,354	1,618	0,261
Putkaste	forest floor	3,859±0,201	1,626±0,145		
Kuusnõmme	soil	1,161±0,004	9,593±0,035	-	-
Kuusnõmme	forest floor	-	-		
Nõva	soil	0,065±0,006	8,857±0,407	58,005	0,125
Nõva	forest floor	3,746±0,249	1,110±0,164		
Värska	soil	0,271±0,014	12,629±0,325	9,007	0,104
Värska	forest floor	2,442±0,22	1,312±0,224		

The highest value was found in the forest floor sample from the Vigala site. The greatest difference between the soil and litter activities was found at site Nõva due to extremely low soil activity. The use of organic matter content in normalisation yielded highest activity values for soils from sites Värska, Kuusnõmme and Vigala. Low activities were recorded for forest floor samples from sites Nõva and Värska. When soil dry weight was used in calculations our results indicate that the microbial activity was higher in forest floor layer on all sites. When organic matter content of substrate (forest floor, soil) was considered then it was possible to depict differences between and within sites (ratio 1 and ratio 2 in table 3). The low activity of microbes in the litter layers of pine forests (sites Nõva and Värska) indicates the effect of substrate type on litter decomposing micro-organisms.

Earthworm community is the most important component of soil fauna attending in the regulation of decomposition and nutrient cycling (Edwards, Bohlen, 1996). All species are participating in decomposition and mixing of the organic and inorganic components but their effect on the soils differs (Edwards, 1985). During the unfavourable period the anecic earthworms live in deep burrows, endogeic live in topsoil, while epigeic earthworms depend on the humidity of the habitat (Bouche, 1977). The number and species composition of earthworm community in different stands was variable (Table 4). In spruce stands the numbers of individuals and species in the Voore study area were highest (116...120 individuals per 1 m², 8 species). Different endogeic and epigeic species of earthworms were found on this areas (*Allolobophora caliginosa, Allolobophora rosea, Lumbricus rubellus, Lumbricus castaneus, Dendrodrilus rubidus, Dendrobaena octaedra*). Timm

Table 4. Number of individuals and species of earthworms in the forest floor and in the soil of study sites, in October 1997.

		Species*							
Site	Fraction	*A.cal*	*A.ros*	*A.chl*	*L.rub*	*L.cas*	*D.rub*	*D.oct*	Total
		Number of ind. per 1 m² (mean±SE)							
Voore	forest floor	12±2	4±2	0	12±5	4±1	56±9	0	88±28
	soil	20±10	4±1	0	4±1	0	0	0	28±10
Vigala	forest floor	3±2	0	0	4±1	0	24±4	4±2	37±9
	soil	0	0	0	0	0	0	0	0
Putkaste	forest floor	0	0	0	8±2	12±2	28±5	0	48±11
	soil	6±2	2±1	0	0	0	0	0	8±2
Kuusnõmme	forest floor	4±1	0	0	8±2	2±1	6±2	0	20±3
	soil	84±9	12±5	8±4	0	0	0	0	104±7
Värska	forest floor	0	0	0	0	0	0	0	0
	soil	0	0	0	0	0	0	0	0
Nõva	forest floor	0	0	0	0	0	8±4	0	8±4
	soil	0	0	0	0	0	0	0	0

* *A.cal – Allolobophora caliginosa*
A.ros – Allolobophora rosea
A.chl – Allolobophora chlorotica
L.rub – Lumbricus rubellus
L.cas – Lumbricus castaneus
D.rub – Dendrodrilus rubidus
D.oct – Dendrobaena octoedra

and Frey (1979) have studied the earthworms in spruce forests in Estonia and for the Voore study site, they have published the number of individuals (50...190 individuals per m²) and species (7). On the study area at Vigala the number of earthworms was low (15...33) and we found only 3 epigeic species (*Dendrodrilus rubidus, Lumbricus rubellus, Dendrobaena octaedra*) and 1 endogeic species (*Allolobophora rosea*) in forest floor. On the Putkaste study area, 3 epigeic species (*Dendrodrilus rubidus, Lumbricus rubellus, Lumbricus castaneus)* in forest floor and 2 endogeic species (*Allolobophora caliginosa, Allolobophora rosea*) in mineral soil were found. On the study area at Kuusnõmme with abundant grass layer, the numbers of earthworm individuals and endogeic species were large, and the typ-

ical species of grasslands dominated (*Allolobophora caliginosa, Allolobophora chlorotica, Allolobophora rosea, Lumbricus rubellus*). In pine stands the forest floor was quite acidic and only few earthworm species tolerate such conditions. After Lee (1985), the earthworms are not able to live in medium with pH<3.5, and the pH range 3,5...4,5 is suitable for few epigeic earthworm species (*Dendrodrilus rubidus)*. On the Värska study area where the pH of the forest floor and soil were low (3,6...4,4) no earthworms were found during the year. A very small number of earthworms was found in forest floor layer (*Dendrodrilus rubidus, Allolobophora caliginosa*) on the Nõva study site.

There was found significant linear relationships for soil between the number of species and pH (r = 0,86) and number of individuals per 1 m² and pH (r = 0,91), respectively. For forest floor significant multiplicative relationships between the number of species and pH (r = 0,94) and number of individuals per 1 m² and pH (r = 0,93) were found. The level of significance p<0,0001 in all cases. The effect of the nitrogen content, organic matter content and soluble phosphorus content of forest floor and soil on the earthworm community was statistically insignificant.

Conclusions

1. The number of earthworm individuals (0...88) and species (0...5) was variable in forest floor of coniferous forests. The number of earthworm individuals and species in mineral soil of all studied forests was 0...28 and 0...3, respectively, and 104±7 individuals per m² and 7 species, as exception, in the spruce forest with abundant grass layer.

2. In spruce forests the number of earthworm individuals was 15...124 per 1 m² and the number of species was 2...8, in pine forests the numbers were: 0...8 individuals per m² and 0...2 species.

3. The mean microbial activity in the forest floor of spruce forests was 4,636±1,04 OD/g, that of pine forests 3,094±0,69 OD/g (or, 2,056±0,26 OD/OM and 1,211±0,10 OD/OM, respectively). The mean microbial activity in the soil of the spruce forests was 1,855±0,55 OD/g and that of the pine forests 0,168±0,10 OD/g, (or, 8,418±0,82 OD/OM and 10,743±1,89 OD/OM, respectively).

4. The significant linear relationships for soil between the number of species and pH (r = 0,86) and number of individuals per 1 m² and pH (r = 0,91) in soil, significant multiplicative relationships between the number of species and pH (r = 0,94) and number of individuals per 1 m² and pH (r = 0,93) in forest floor were found

Acknowledgements

Support for this work came from grant No 2726 from Estonian Science Foundation.

Reference

Bouche M. B. 1977. Strategies lombriciennes. Soil Organisms as components of ecosystem (eds. U.Lohm, T.Persson). Ecol.Bull. (Stockholm), 25: 122-132

Edwards C. A. 1985. Earthworms in soil formation, structure and fertility. Quaest. Entomol., 21: 517-522.

Edwards C. A. and Bohlen P. J. 1996. Biology and Ecology of Earthworms. 3rd edition. London, Chapman & Hall, 426 pp.

Görres J. H., Dichiaro M. J., Lyons J. B. and Amador J. A. 1998. Spatial and temporal patterns of soil biological activity in a forest and an old field. Soil Biology and Biochemistry, 30:219-230

Johansson M. B. 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in Scandinavian forests. Forestry, vol. 68: 49-62.

Lee K. E. 1985. Earthworms. Their Ecology and Relationships with soils and Land Use. London, Ac. Press, 388 p.

Lõhmus K., Ivask M., Ostonen I. 1995. Decomposition of fine roots of Norway spruce (*Picea abies* (L.)Karst.) and scots pine (*Pinus silvestris* L.) in different soils. Role of roots, mycorrhizas and rhizosphere in carbon cycling in forest soil. Finnish Forest Research Institute, Research Papers 537, Helsinki: 83-87.

Nordström S., Rundgren S. 1973. Associations of lumbricids in Southern Sweden. Pedobiologia 13: 301-326

Rutigliano F. A., De Santo A. V., Berg B., Alfani A, Fioretta A. 1996. Lignin decomposition in decaying leaves of *Fagus sylvatica* L. and needles of *Abies alba* Mill. Soil Biol. Biochem., 28: 101-106

Saetre P. 1998. Soil organisms, Ground vegetation and ecosystem processes in mixed stands of Norway Spruce and Birch. Acta Universitatis Agriculturae Succiae, Silvestria 54. Doctor's dissertation, Uppsala.

Satchell J. E. 1967. Methods of sampling earthworm populations. Pedobiologia, 9: 20-25.

Schnürer J., Rosswall T. 1985. Fluorescein hyrdolysis as a measure of total microbial activity in the soil and litter. Appl. Env. Microbiol., 43: 1256-1261.

Timm T., Frey T. 1979. A note on earthworms (*Lumbricidae*) on spruce forests. In: Spruce forest ecosystem structure and ecology. 2. Basic Data on the Estonian Vooremaa Project (ed.by T.Frey). Tartu, 101-103

СТРУКТУРА И АКТИВНОСТЬ СООБЩЕСТВ МИКРООРГАНИЗМОВ И ДОЖДЕВЫХ ЧЕРВЕЙ В ХВОЙНЫХ ЛЕСАХ ЭСТОНИИ

М. Иваск, Я. Труу, М. Труу, К. Лыхмус, И. Остонен

Резюме

Изучены сообщества разлагателей в лесной подстилке и в гумусевом горизонте в ельниках и сосняках. Структура сообщества дождевых червей зависит от свойств лесной подстилки. В то же время, травянистая растительность также влияет на численность червей и разнообразия их видов. В ельниках, численность дождевых червей намного выше чем в сосняках, 15...124 и 0...8 особей на 1 м², соответственно. Число видов также было выше в ельниках по сравнений с числом видов в сосняках (2...8 и 0...2, соответственно). Активность сообщества микроорганизмов в ельниках выше чем в сосняках.

Ключевые слова: сообщества дождевых червей, лесная подстилка, активность микроорганизмов, ель европейская, сосна обыкновенная.

IV

Ivask M, Lõhmus K, Truu J, Truu M, Ostonen I. 2000.
Earthworm Lumbricidae communities in alder and aspen forest: three case studies.
Baltic Forestry 6, 1, 74–77.

Earthworm *Lumbricidae* Community in Alder and Aspen Forest: Three Case Studies

MARI IVASK, JAAK TRUU, MARIKA TRUU
Environmental Protection Institute, Estonian Agricultural University
Kreutzwaldi 5, 51014 Tartu, Estonia
KRISTA LÕHMUS
Institute of Geography, University of Tartu
Vanemuise 46, 51014 Tartu, Estonia
IVIKA OSTONEN
Institute of Botany and Ecology, University of Tartu
Lai 40, 51005 Tartu, Estonia

Ivask M., Truu J., Truu M., Lõhmus K., Ostonen I. 2000. Earthworm *Lumbricidae* Community in Alder and Aspen Forest: Three Case Studies. *Baltic Forestry*. 1: 74–77.

The earthworm community structure was investigated in soil and forest floor of three deciduous forests (*Aegopodium* type). The study areas for investigation of decomposers were: aspen forest in Kärkna Forestry District; grey alder forest on Porijõgi catchment area (unpolluted area); and grey alder forest in Viljandi Forestry District, heavily polluted by pig slurry. All three study areas were characterized by optimal for investigated earthworm species moisture content (24,6...61,2%). In grey alder and aspen forests the number of individuals and species of earthworms per 1 m² is high (135...309 individuals, 6...7 species per 1 m²) due to the character of litter and soil. The common species in aspen and grey alder forests were *Allolobophora caliginosa, Allolobophora rosea, Lumbricus rubellus, Dendrodrilus rubidus*. The species composition of decomposer community was affected by contamination of forest soil with pig slurry, some of earthworm species (*Allolobophora chlorotica, Lumbricus castaneus*) were not found in slurry-contaminated forest floor and soil.

Key words: earthworm community, aspen forest, grey alder forest, pig slurry.

Introduction

The alder forests are typical riparian ecosystems in agricultural landscapes, what are evaluated as buffer zones to protect water bodies against pollution (Mander et al., 1997). Due to their rapid growth, grey alder (*Alnus incana* (L.)Moench. and aspen (*Populus tremula* L.) are also perspective indigenous short rotation species in Baltic countries (Tullus et al., 1996). For environmental reasons and sustainable management of buffer stands, it is important to study the decomposition of litter from grey alder and aspen forests. The litter decomposition process is affected by many factors, among them by soil organisms - decomposers. Biomass and activity of decomposers largely control the rates of mineralization and turnover of organic matter (Brown, 1995). The soil fauna taxonomically is very diverse. The earthworms are the most important group of soil fauna for decomposition process because of burrowing activity and size (White, 1987). In deciduous forests the most important species of decomposers are: endogeic species *Allolobophora caliginosa* and *Allolobophora rosea*, epigeic species *Dendrodrilus rubidus* and *Lumbricus rubellus* (Bell 1974). By contamination with slurry the communities of the soil fauna are strongly influenced, the species of earthworms tolerate it differently (Andersen, 1981).

The aim of present study was to analyse the structure of communities of earthworms in three deciduous forests (*Aegopodium* type) in Estonia: aspen forest and two grey alder forests, one of them heavily contaminated by pig slurry.

Material and methods

Study areas for investigation of litter decomposers were:

1. Aspen forest in Kärkna (aged 42 years, *Aegopodium* type, good developed herb layer) description of the area is published (Tullus and Tamm, 1996).

2. Grey alder forest in Porijõgi location (aged 16 years, *Aegopodium* type, unpolluted area), description of the area is published (Mander et al., 1995).

3. Grey alder forest in Viiratsi location (aged 41 years, *Aegopodium* type, heavily polluted by pig slurry), description of the area is published (Mander et al., 1997).

The forest floor and soil samples for chemical and microbiological analysis were collected in May and June, on the quadrates 50 x 50 cm or on the rings Ø 104 mm. The number of sample areas varied from 10 to 22. Samples were cleaned, sorted, weighed and dried at 75°C. The oven-dry weight and moisture content in all samples, and organic matter content in composite sample (in muffel oven at 360°C) were determined. Soil samples were taken on the same quadrates or the rings, in the upper soil layer 0...15 cm. In all samples the moisture content, in a composite sample the organic matter content (in muffel oven at 360°C) and nitrogen concentration (by the Kjeldahl method) were determined.

Earthworm samples were collected in May and October at the time of maximum density, greatest activity and lowest variability of individuals (Nordström and Rundgren, 1973). Earthworms were collected from soil blocks measuring 50 x 50 x 40 cm by hand sorting (Satchell, 1967), separately from forest floor and soil; they were washed and identified to species. All earthworms were divided into three ecological groups (Bouche, 1977): 1. Epigeics – living in the soil surface (*Lumbricus rubellus, Lumbricus castaneus, Dendrodrilus rubidus*); 2. Anecics – forming deep burrows (*Lumbricus terrestris*); 3. Endogeics – inhabits of mineral soil horizons (*Allolobophora caliginosa, Allolobophora rosea, Allolobophora chlorotica, Eisenia foetida*).

For all sampling occasions and earthworm species the mean numbers of individuals per 1 m^2 and their standard errors were calculated. One-way analysis of variance and the multiple comparison of means were used for comparison of different communities at the level of significance $p<0,05$.

Results and discussion

Earthworms community is the most important component of the soil fauna attending in the regulation of decomposition and nutrient cycling. All earthworm species are participating in decomposition and mixing the organic and inorganic components (Edwards, 1985). During the unfavourable (cold, drought, flooding) period the endogeic earthworm species live in the soil, while epigeic earthworms are more dependent on the moisture of their habitat (Brown, 1995). They can use a wide variety of organic materials for food and most species of earthworms can distinguish between different kinds of forest litter (Edwards and Bohlen, 1996). Leaf litter of aspen and grey alder is attractive for earthworms and litter with a high protein content is more readily accepted by earthworms (Satchell, 1967, Edwards and Bohlen, 1996). The nitrogen content of forest floor was 2,15...3,28% in grey alder forests and 1,05% in aspen forest, nitrogen content of the soil was 0,7...1,05 and 0,26, respectively. The mean moisture content in the soil of investigated stands (24,6...61,2%) (Table 1) was optimal for earthworm species (Edwards and Bohlen, 1996).

Table 1. Parameters of forest floor and soil of study areas

Parameter	Aspen forest	Grey alder forest (non-contaminated)	Grey alder forest (contaminated)
Number of samples	16	10	22
Soil moisture content, % (mean±SE)	24,6±0,7	61,2±1,7	36,5±1,6
Dry weight of forest floor per 1 m^2, g (mean±SE)	625±85	193±17	43±14

In the Kärkna study area the herb layer under aspen forest is well developed. The common earthworm species (*Allolobophora caliginosa, Allolobophora rosea, Lumbricus rubellus, Dendrodrilus rubidus*) and typical species of grasslands (*Allolobophora chlorotica, Lumbricus castaneus*) were found in this forest. 87...95% of collected earthworms were endogeic and only few individuals (*Dendrodrilus rubidus*) were epigeic (Table 2). In the Porijõgi study area, in non-contaminated grey alder stand the number of individuals (309±24 in May and 232±51 in October) and species (6...7) was high. The reason for this can be nitrogen concentration of litter 2,15...3,28%, indicating high protein content. The species composition in non-contaminated grey alder forest was similar to species living in the soil and forest floor of aspen stand (Table 3). 27...40% of individuals were epigeic.

The total number of individuals was higher in grey alder stand without pollution pressure than in contam-

Table 2. The parameters of earthworm communities

Stand	Material	May		October	
		Individuals per 1 m² (mean±SE)*	Species ocurred on sample area	Individuals per 1 m² (mean±SE)*	Species ocurred on sample area
Aspen forest	forest floor	25±4 [a]	6	8±3 [a]	1
	soil	168±12 [b]	5	176±33 [b]	6
	total	193±42 [b]	6	184±16 [b]	7
Grey alder forest (non-contaminated)	forest floor	29±14 [a]	5	-	-
	soil	280±22 [c]	7	232±51 [b]	6
	total	309±24 [c]	7	232±51 [b]	6
Grey alder forest (contaminated)	forest floor	8±2 [a]	2	-	-
	soil	127±15 [b]	6	168±20 [b]	6
	total	135±18 [b]	6	168±20 [b]	6

* numbers followed by the same letter does not significantly differ (multiple comparison of means, $p<0,05$)

Table 3. Species composition of earthworm communities in deciduous forests in October 1997 (number and % of individuals, collected from forest floor and soil).

Species	Aspen forest		Grey alder forest (non-contaminated)		Grey alder forest (contaminated)	
	Number (mean±SE)	%	Number (mean±SE)	%	Number (mean±SE)	%
Allolobophora caliginosa	111,0±7,1	60,3	136,9±49,7	59,0	108,0±16,0	64,3
Allolobophora rosea	13,3±1,3	7,2	21,3±14,1	9,2	9,3±3,5	5,5
Allolobophora chlorotica	16,0±8,5	8,7	18,7 ±7,1	8,1	-	-
Lumbricus rubellus	6,7±1,3	3,6	9,8±4,5	4,2	6,7±0,9	4,0
Lumbricus castaneus	1,3±1,0	0,7	16,0±9,2	6,9	-	-
Lumbricus terrestris	-	-	-	-	5,3±1,3	3,2
Dendrodrilus rubidus	6,7±4,8	3,6	24,0±6,1	10,3	7,7±9,3	4,6
Eisenia foetida	-	-	-	-	8,0±2,3	4,8
undetermined	29,0±4,8	15,9	5,3±1,8	2,3	23,0±13,3	13,6
Total	184±16	100,0	232±51	100,0	168±20	100,0

inated one (309±24 and 135±18 in May, respectively, and 232±51 and 168±20 in October, respectively) (Table 2). The structure of earthworm community in contaminated grey-alder stand differed significantly ($p<0,001$) from that in clean grey-alder stand. The grassland species were not found because they are not able to live in the soil contaminated by slurry. 8,6...10,5% of individuals were epigeic and 1,9...4,5% were anecic. The typical inhabitant of decaying manure and compost, *Eisenia foetida*, was present.

All the same species of earthworms were found in clean forests; in our cases tree species was not an important factor for community structure. By comparing the total numbers of individuals per 1 m² of three deciduous forests the statistically significant difference between non-contaminated forests and contaminated grey alder forest was found (one-way-analysis of variance, the level of significance $p<0,05$).

The common earthworm species (endogeic *Allolobophora caliginosa*, *Allolobophora rosea* and epigeic *Lumbricus rubellus*) were living in all three stands. Species *Allolobophora chlorotica* lived only in clean habitats. Epigeic species *Lumbricus castaneus* was found in non-contaminated areas only, but the difference between the numbers of individuals per 1 m² was statistically insignificant. Two species (anecic *Lumbricus terrestris* and endogeic *Eisenia foetida)* were found only in contaminated forest. The numbers of individuals per 1 m² of *Eisenia foetida, Lumbricus terrestris* and *Allolobophora chlorotica* were different in contaminated and non-contaminated stands, significance levels were $p<0,001$, $p<0,005$ and $p<0,05$, respectively.

Conclusions

1. In grey alder and aspen forests (*Aegopodium* type) the number of earthworms (135...309 individuals per 1 m²) and species (from 12 species, found in Estonia, 8 were presented) is high.

2. Earthworm species composition was changed and total number of individuals decreased in the floor and litter of grey alder forest contaminated with pig slurry.

Acknowledgements

This work was supported by grant No 2726 from Estonian Science Foundation.

References

Andersen C. 1981. Nitrogen turnover by earthworms and related problems in animal manured plots. Nord. jordbrugsforsk., 63, 2: 363-364

Bell M. 1974. Herbaceous litter. In: Biology of plant litter decomposition. Ed.by Dickinson and Pugh. Ac.Press.

Bouche M. B. 1977. Strategies lombriciennes. Soil Organisms as components of ecosystem (eds. U.Lohm, T.Persson). Ecol.Bull. (Stockholm), 25: 122-132

Brown G. G. 1995. How do earthworms affect microfloral and faunal community diversity? - The significance and regulation of soil biodiversity. H.P.Collins, G.P.Robertson, M.J.Klug (eds.) Kluwer, pp.247-269.

Edwards C. A. 1985. Earthworms in soil formation, structure and fertility. Quaest. Entomol., 21: 517-522.

Edwards C. A. and Bohlen P. J. 1996. Biology and Ecology of Earthworms. 3rd edition. London, Chapman & Hall, 426 pp.

Mander Ü., Kuusemets V. and Ivask M. 1995. Nutrient dynamics of riparian ecotones: a case study from the Porijõgi River catchment, Estonia. Landscape and Urban Planning, 1995,31: 333...348.

Mander Ü., Lõhmus K., Kuusemets V. and Ivask M. 1997. The potential role of wet meadows and grey alder forests as buffer zones. In: Haycock N. E., Burt T. P., Goulding K. W. T., Pinay G. (Eds.) Buffer Zones, Their Processes and Potential in Water Protection. Proceedings of the International Conference on Buffer Zones, September 1996. Part II. Oxford, Quest Environmental, Foundation for Water Research, pp. 35...46.

Nordström S. and Rundgren S. 1973. Associations of lumbricids in Southern Sweden. Pedobiologia 13: 301-326

Satchell J. E. 1967. Lumbricidae. Soil Biology (eds. A.Burgess, F.Raw), Academic Press, London, pp.259-322

Tullus H., Keedus K., Uri V., Mander Ü. and Lõhmus K. 1996. Sustainable forest management in grey alder stands as energy and buffer forests in Estonia. In: Planning and implementing forest operations to achieve sustainable forests. IUFRO 19th Annual Meeting, July 29-August 1, 1996, Marquette, Michigan USA (Ch. R. Blinn, M. A. Thompson, eds.), pp.92-101

Tullus H. and Tamm Ü. 1996. The effect of some factors on the thickness of aspen leaves. (Haavalehtede sõltuvus mitmesugustest teguritest). Metsanduslikud uurimused. Tartu, pp.21-35

White R. E. 1987. Introduction to the principles and practice of soil science. 2nd edition. Blackwell Sci, Oxford.

Received 23 December 1998

СООБЩЕСТВА ДОЖДЕВЫХ ЧЕРВЕЙ В ОСИННИКЕ И СЕРООЛЬШАИНИКАХ

М. Иваск, К. Лыхмус, Я. Труу, М. Труу, И. Остонен

Резюме

Структура сообщества дождевых червей исследовалась в лесной подстилке и гумусовом горизонте трёх лиственных лесов: незагрязненных осинника (Кяркна) и сероольшайника (Порийыги) и загрязненного жидким свиным навозом сероольшанника (Вийратси). Влажность почвы всех изученных древостоев была оптимальной для жизни дождевых червей. Вид дерева (серая ольха, осина) не оказал влияние на видовой состав сообщества дождевых червей. Численность особей в лесной подстилке и гумусовом горизонте варьировала в пределах 135-309 экз/м$^{-2}$. Загрязнение жидким навозом оказало влияние на структуру сообщества разлагателей. Некоторые виды (*Allolobophora chlorotica, Lumbricus castaneus*) не выносили загрязнения навозом.

Ключевые слова: дождевые черви, лесная подстилка, гумус, осинник, сероольшанник, загрязнение.

V

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

Plant and Soil **257:** 435–442, 2003.

Proportion of fungal mantle, cortex and stele of ectomycorrhizas in *Picea abies* (L.) Karst. in different soils and site conditions

Ivika Ostonen[1,2,3] & Krista Lõhmus[1]

[1]*Institute of Geography, University of Tartu, Vanemuise 46, 51014, Estonia.* [2]*Institute of Botany and Ecology, University of Tartu, Lai 40, 51005, Estonia.* [3]*Corresponding author**

Received 22 October 2002. Accepted in revised form 15 July 2003

Key words: cortex and stele ratio, ectomycorrhiza, *Picea abies*, short root anatomy, soil conditions

Abstract

Anatomical variability of ectomycorrhizal short roots in Norway spruce (*Picea abies* (L.) Karst) stands was investigated in five stands differing in site quality class (I^a–V) and soil type. Ten root samples per stand were randomly collected from the forest floor and the subsequent 20 cm soil layer. Thin transverse or axial sections (5 μm) of randomly taken short roots were examined by light microscopy (AXIOPHOT; magnification 200–800×). All analyzed short root tips of Norway spruce in different stands were colonized by ectomycorrhizal fungi. Thickness of the mantle (T_{mantle}) and cortex (T_{cortex}) and diameter of the root (D_{root}) were measured in four crossing radial directions for transverse sections and in two radial directions for axial sections. The proportions of the mantle (PS_{mantle}), cortex (PS_{cortex}) and stele (PS_{stele}) of the root cross-sectional area (CSA) were calculated. Mean T_{mantle} and T_{cortex} varied from 16.5 ± 0.6 to 29 ± 1.3 μm and 83.9 ± 1.7 to 108.4 ± 2.4 μm, respectively; significant differences between stands were found. The number of cell rows in the cortex (4–6) did not vary between different stands, thus thickness of cortex depended on cell size. Mean PS_{mantle}, PS_{cortex} and PS_{stele} of the root CSA varied from 17.7 to 28.1%, from 58.9 to 66.9%, and from 13.4 to 15.8%, respectively. No differences between stands were revealed in mean CSA ratio of cortex and stele. It can be concluded that irrespective of big differences in soil and site conditions, including the influence of fungal symbionts, a 4:1 relation on a CSA basis between the cortex and stele is inherent to Norway spruce.

Introduction

The short roots of spruce are as a rule ectomycorrhizal (ECM) roots with primary structure that is functionally adapted for the uptake of water and mineral nutrients. Root anatomy and morphology are genetically controlled but are also influenced by environmental biotic and abiotic factors including soil moisture and nutrients as well as fungal symbionts. A detailed analysis of the anatomical and morphological structure of ectomycorrhizas is necessary in order to begin to understand the importance of their structural parameters in regard to function. Data on structural parameters of short roots and their impact on physiological processes is still scarce. At the same time it has been clearly demonstrated that structural parameters of leaves reflect leaf functioning in relation to different environmental conditions extremely well (Niinemets and Kull, 1998). Strategically, leaves and fine roots have a key position as the most active interfaces between the environment and the plant. Hence, successful growth of a plant strongly depends on structural adaptations of these important organs to changes in the environment. Analogously to the fit of foliar parameters to the environment, environmental-induced structural parameters of fine roots and ectomycorrhizas also occur (Comas et al., 2002; Fitter, 1985; Lõhmus et al., 1989; Ostonen et al., 1999; Persson et al., 1998). Our previous study (Ostonen et al., 1999) about variation of morphological parameters of Norway spruce ECM short roots in different soil conditions indicated that the most important soil characteristics related to root

* FAX No: +372-7-375-825; E-mail:ivika@ut.ee.

morphology were the humus content, field capacity and specific soil surface area; eleven measured soil variables explained 28% of the total variance of the root morphological characteristics, and were related to site optimality.

Considering an ECM root, it consists spatially and functionally of three different parts: stele, cortex and fungal mantle. The first is responsible mainly for the long-distance bi-directional transport within the plant. At the same time, the endoderm surrounding the stele forms an almost impermeable barrier separating long-distance and short-distance transport. The structure and function of the two other components, (cortex and mantle) is more complicated, because they form a transition zone between plant and fungus (cortex and Hartig net), and plant and soil (mantle and/or rhizodermal cells of cortex). The Hartig net forms a highly branched network in the apoplast of the root cortex and constitutes the interface for the exchange of photoassimilates, soil water and nutrients between the host plant and its fungal partner. There are few studies that have examined the amount of fungal biomass in fine roots in relation to soil conditions (Ekbald et al., 1995; Hobbie and Colpaert, 2003). The mantle is the transition zone between the plant and the soil and its structure and thickness varies greatly depending on the plant and fungal species forming the symbiosis (Agerer, 1987–97) and on environmental conditions (Kåren, 1997). As microfilms of bacteria are abundant in the soil-root interface, including hyphae in the mantle (Sarand et al., 1998), the mantle is a zone of intensive interactions between plant and soil microbiota.

Horton and Bruns (2001) and Read and Perez-Moreno (2003) have given overviews describing remarkable progress in ECM ecology; they report findings on the general characteristics of ECM communities (their structure, dynamics and diversity) and on the impact of sampling strategies on investigated parameters (Taylor, 2002). Recent studies regarding the role of ECM in plant nutrition have shown that some ECM fungi are able to mobilise organically bound nutrients from litter (Perez-Moreno and Read, 2000) and even capture nutrients from saprotrophic fungi (Lindahl et al., 1999). Ectomycorrhizas produce prodigious amounts of hyphae, which extend from the surface of the mantle into the soil, and are responsible for much of the nutrient uptake in ECM plants (Brandes et al., 1998) as well as for the transfer of photosynthates to drive soil respiration (Högberg et al., 2001). Simard et al. (2002) made a calculation based on average hypha and mycorrhiza diameter, that hyphae produce up to a 60-fold increase in surface area of mycorrhizal fine roots. It is known that ECM species vary in their ability to acquire specific nutrients from the soil (Leake and Read, 1997).

As short root development in Norway spruce is influenced by soil properties and ECM symbiosis, there is a need to determine the natural variability of short root anatomy and morphology in relation to soil conditions and fungal symbionts. Furthermore, mycorrhizal fine root turnover accounts for a considerable amount of carbon in the ecosystem carbon cycle. Information on the carbon cost of the fungal partner incorporated into ECM tissues is still scarce (Hobbie and Colpaert, 2003; Lindahl et al., 2002; Rousseau and Reid, 1989). Hence, there is an urgent need to assess the dimensions and proportions of the stele, cortex and mantle of ectomycorrhizas in relation to soil and site conditions.

Our main objectives were (1) to estimate the share of stele, cortex, and mantel in ECM short roots in Norway spruce in different sites, (2) to analyze the pattern of anatomical variability of ectomycorrhizas in different soil and site conditions.

Materials and methods

Site descriptions

The study was carried out on five permanent plots of 50 × 50 m established in 1987 in coniferous stands located throughout Estonia (Table 1). Each stand represents a particular forest type, site quality class, and soil type. Stand and soil characteristics have been described more detailed by Lõhmus and Ivask (1995) and Ostonen et al. (1999).

Sampling and processing of roots

Earlier background investigations of short root morphological parameters – root tissue density (RTD, mg mm^{-3}; [(1)] and specific root area (SRA, mm^2 mg^{-1}; [(2)] are described in Ostonen et al. (1999), where, for RTD, we used the term 'root density' (RD) and Lõhmus et al. (1989). In the literature root density commonly indicates the amount of roots per unit of soil volume or study site area; this term may be confusing unless it is used in the correct sense. Use of the term is considered in the Discussion. A more frequently used root parameter is also specific root length (SRL, mm mg^{-1}; (3)) (Ford and Deans,

Table 1. Characteristics of the Norway spruce sites in Estonia

Area, location	Forest site type	Age years	Site quality class	Soil type	H %	Field capacity mm	pH H_2O
Roela 58° 42′ N; 26° 45′ E	*Oxalis* spruce forest	59	I[a]	Umbric Luvisol	4.05	33.89	5.75
Putkaste 58° 49′ N; 22° 33′ E	*Hepatica* spruce forest (drained)	74	II	Eutri Mollic Gleysol	8.5	30.34	5.7
Vigala 58° 46′ N; 24° 15′,E	*Oxalis* spruce forest (drained)	53	I	Dystric Gleysol	9.3	32.72	4.75
Kuusnõmme 58° 19′ N; 21° 59′ E	*Calamagrostis* spruce forest	83	IV–V	Rendzic Leptosol	3.71	34.14	7.7
Pikasilla 58° 05′ N; 26° 03′ E	*Vaccinum vitis-idea* pine forest	73	III	Sombri-Ferric Podzol	2.65	28.86	5.0

H – humus content.

1977; Fitter, 1985; Majdi, 1994; Persson et al., 1998; Pregitzer et al., 2002). The parameters described are calculated as follows:

RTD = root dry weight / root volume, (1)

SRA = root surface area / root dry weight, (2)

SRL = root length / root dry weight, (3)

For investigation of short root anatomical characteristics, in October 1997, 10 root samples were randomly collected in each study plot, from the forest floor and, by spading, from the 20 cm thick soil surface layer where 80–90% of fine roots are situated (Lõhmus and Lasn, 1990). The excavated samples were gathered into a composite sample and root fragments were not separated according to soil horizons, since our earlier data revealed no difference in the SRA of ectomycorrhizas in different soil layers (Lõhmus et al., 1989). Roots were washed with tap water and a small soft brush to remove all soil particles. Thin transverse or axial sections 5 (μm) of randomly taken short roots were cut using the freezing microtome cryostat Microm (HM 500 OM, –21 °C). The embedding medium for frozen tissue specimens was TISSUE-TEC (O. C. T. 4583 compound, MILES-USA). All sections were coloured with methylene-blue and stained in 'Mount-Quik-Aqueous'. Root sections were examined by light microscopy (AXIOPHOT; magnification 200–800×). One hundred and ten to 176 (total 744) sections were measured per each spruce forest area. Short root anatomical characteristics: thickness of the mantle (T_{mantle}) and cortex (T_{cortex}), and the diameter (D_{root}) of a transverse root section were measured in four crossing radial directions; measurements on axial sections were performed in two radial directions. The diameter of the stele (D_{stele}) and proportions D_{stele}/D_{root} and $D_{stele}/D_{stele+cortex}$ were calculated. The percentages of the root cross sectional area (CSA), of the mantle, cortex, and stele were calculated as follows:

$$PS_{mantle} = 100 * S_{mantle} / S_{root}, \quad (4)$$

$$PS_{cortex} = 100 * S_{cortex} / S_{root}, \quad (5)$$

$$PS_{stele} = 100 * S_{stele} / S_{root}, \quad (6)$$

where S = area of root and its compartments.

Specific endoderm area, using data (SRL) from our previous study (Ostonen et al., 1999) was calculated as follows:

$$SEA = \pi D_{stele} SRL \quad (7)$$

$$SEA = (D_{stele} / D_{root}) SRA \quad (8)$$

Statistics

Except for the PS_{cortex} and SEA, the root parameters were not normally distributed. To normalize the variables, root diameter and T_{cortex} were log transformed, T_{mantle}, PS_{mantle}, PS_{stele} and D_{stele} were repeatedly square-root, log or arcsine transformed. However, group variances of PS_{mantle} and PS_{stele} were inhomogenous and the group means were compared by 95%-confidence intervals. For the rest of parameters multiple comparison of means was applied using Tukey test for unequal n.

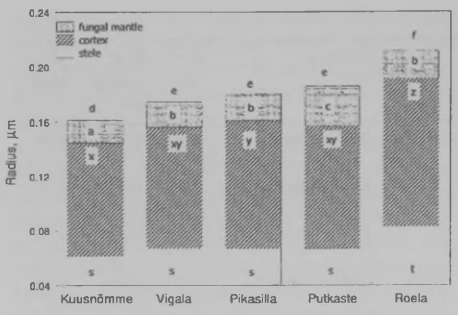

Figure 1. Mean characteristics of Norway spruce short roots in five study areas. Letters: a, b, c denote significant differences between mean thickness of fungal mantle; x, y, z between mean thickness of cortex; s, t between mean of stele radius and d, e, f, between means of root radius.

Nonparametric Gamma correlation coefficients were calculated to estimate strength and significance of relationship as one variable (site quality class) was in ordinal scale and the data contained many tied observations. In all cases, level of significance $\alpha = 0.05$ was accepted.

Results

Root variability

For all study areas (Table 1), variability of root diameter as well as variability of subsequent functionally different compartments of ECM root: fungal sheath and cortex, including Hartig net and stele, were investigated. Stand means for thickness of fungal mantle and cortex, stele diameter, and root diameter are presented in Table 2. Significant differences are indicated by different letters in Figure 1. Variability within a stand, expressed as relative standard error (standard error/mean), was less than 5% for all variables in Table 2, except thickness of fungal mantle.

Mean proportions of fungal mantle (PS_{mantle}), cortex (PS_{cortex}),and stele (PS_{stele}) of short root CSA were calculated for each stand (Table 3) and their values were in the following order: $PS_{cortex} > PS_{mantle} > PS_{stele}$. To exclude the variability inherent to the fungal sheath, the proportions of stele and cortex were calculated on an area basis (Table 3).

The variability of the fungal sheath and Hartig net

All analyzed short root tips of Norway spruce in different stands were colonized by ECM fungi; the mantle and the Hartig net were found in all cases. The Hartig net was mostly of uniform thickness throughout the cortex. Only in the Vigala spruce stand (Table 1), were some root cross-sections detected where the Hartig net penetrated only through the outer cell layers. The fungal mantle was discontinuous for some short roots collected from the Roela study area. In all cases, no root hairs were observed. The structure of the mantle varied from smooth to loose and the colour from white to black. On the basis of macroscopic features of ectomycorrhizas (color, structure of mantle surface), differences between stands were observed. The number of ectomycorrhizas with a loose mantle was greatest in Roela.

Thickness of the fungal mantle varied between different ectomycorrhiza morphotypes; in all investigated spruce stands the values varied from 0 to 70 μm. Mean thickness of fungal mantle ($\pm$ standard error) varied from 16.5 $\pm$ 0.6 μm to 29.0 $\pm$ 1.3 μm (Table 2), being significantly larger in Putkaste. The mean PS_{mantle} of the root CSA varied from 18 to 28% and was significantly higher in Putkaste than in other stands (Table 3). The highest percentage of ectomycorrhizas with black mantle (the majority, according to macroscopic features, most probably being *Cenococcum*) was also observed in Putkaste; the characteristic varied in different stands from 4.1% (Vigala) to 31.7% (Putkaste).

The variability of cortex

The thickness of the cortex in all investigated stands varied from 39 to 196 μm. The number of cell rows in the cortex (4–6) did not vary between different stands. Mean thickness of the cortex varied from 83.9 $\pm$ 1.8 to 108.4$\pm$ 1.9 μm . The thickest cortex was measured in the highly productive *Oxalis* spruce stand (Roela), the thinnest mean cortex was found in the alvar (*Calamagrostis*) spruce forest of low productivity (Kuusnõmme; Table 2). The mean PS_{cortex} of ectomycorrhizas in investigated stands varied from 59 to 67% and was significantly smaller in Putkaste than in other areas (Table 3). The mean proportion of cortex in the CSA of cortex+stele (mantle excluded), $S_{cortex}/S_{cortex+stele}$ varied from 81 to 82% (Table 3); no statistically significant differences between stands were found.

Table 2. Mean anatomical and morphological characteristics of short roots (mean ± standard error). Significant differences between study areas (Tukey test, $p<0.05$) are indicated by different letters

Area	Thickness of fungal mantle; μm	Thickness of cortex μm	Stele diameter μm	Root diameter μm
Roela	20.5 ± 1.2^{b}	108.4 ± 2.4^{z}	165.6 ± 3.9^{p}	422.5 ± 6.5^{c}
Putkaste	29.0 ± 1.3^{c}	90.0 ± 1.9^{xy}	134.0 ± 4.7^{s}	370.0 ± 7.7^{b}
Vigala	18.6 ± 0.7^{b}	89.0 ± 1.9^{xy}	134.5 ± 3.9^{s}	342.2 ± 6.0^{b}
Kuusnõmme	16.5 ± 0.6^{a}	83.9 ± 1.7^{x}	122.3 ± 3.8^{r}	315.7 ± 5.7^{a}
Pikasilla	19.6 ± 1.0^{b}	93.5 ± 2.3^{y}	134.5 ± 4.8^{s}	361.2 ± 7.3^{b}

Table 3. Mean proportions (%) of fungal mantle, cortex and stele of short root cross-sectional area and mean proportions (%) of plant tissues of Norway spruce short roots (fungal mantle excluded). Significant differences (Tukey test, $p<0.05$) are indicated by different letters

Area	Mantle included			Mantle exluded	
	PS_{mantle} %	PS_{cortex} %	PS_{stele} %	Cortex %	Stele %
Roela	17.7 ± 1.0^{a}	66.9 ± 1.0^{x}	14.9 ± 0.7	81.6 ± 0.8	18.4 ± 0.8
Putkaste	28.1 ± 1.0^{b}	58.9 ± 0.9^{y}	13.4 ± 0.7	81.6 ± 0.8	18.4 ± 0.8
Vigala	21.3 ± 0.8^{a}	64.9 ± 0.8^{x}	14.9 ± 0.6	81.7 ± 0.8	18.3 ± 0.7
Kuusnõmme	19.5 ± 0.7^{a}	65.8 ± 0.7^{x}	15.2 ± 0.6	81.6 ± 0.7	18.4 ± 0.6
Pikasilla	19.9 ± 0.9^{a}	65.2 ± 0.8^{x}	15.8 ± 0.6	80.8 ± 0.7	19.2 ± 0.7

Table 4. The mean specific endoderm area (SEA) of short roots of Norway spruce. Letters denote significant differences between stands

Area	SEA $mm^2\ mg^{-1}$
Roela	20.8^{a}
Putkaste	18.3^{a}
Vigala	20.1^{a}
Kuusnõmme	13.7^{b}
Pikasilla	15.7^{b}

The variability of stele

Stele diameter varied from 15 μm to 358 μm for all measured cross-sections in different stands. Mean diameter of stele varied from 122.3 ± 3.8 μm to 165.6 ± 3.9 μm, D_{stele} was significantly higher in Roela high productivity spruce stand than in other stands (Figure 1). Proportions D_{stele}/D_{root} and $D_{stele}/D_{stele+cortex}$ varied from 35.8 to 39.0% and from 41.4 to 43.1%, respectively; the differences between stands were insignificant. SEA varied from 13.7 to 20.8 $mm^2\ mg^{-1}$ and statistically significant differences between study areas were found (Table 4).

The mean percentage of the stele (PS_{stele}) of the CSA of ectomycorrhizas varied from 13% to 17%. The mean proportion of stele in the CSA of cortex+stele (mantle excluded), $S_{stele}/S_{stele+cortex}$ varied from 18 to 19%; no statistically significant differences between stands were found for either characteristic.

Impact of site quality on dimensions of mantle, cortex and stele

There highly significant ($p<0.000001$) but weak negative correlations were found between dimensions (T_{cortex}, D_{stele}, D_{root}) and site quality class (Table 1); as smaller values of site quality class correspond to higher site fertility, the Gamma rank correlations were –0.22, –0.20 and –0.26 for thickness of the cortex, diameter of stele, and root diameter, respectively. No significant correlation was found between T_{mantle} and site quality class.

Discussion

Since all of the short roots of Norway spruce were ECM, the effects of the fungal symbiont on short-root anatomical-morphological structure appeared in all cases. Hence, significant increase in effective membrane surface area through invagination of mycorrhizal fungi occurred, which has critical implications for efficient root uptake of water and nutrients (Dahlberg, 2001). On the other hand, movement of carbohydrates and nutrients is bi-directional and the impact of roots on the soil is largely mediated by fungal symbionts. The number of different species and morpological types of ECM fungi found in the roots of Norway spruce can be quite high (Egli et al., 1993; Söderström and Bååth, 1978), but the general rule is that a few common ECM species account for most, (>50%), of the mycorrhizal abundance and are widely spread, whereas the majority of species are only rarely encountered (Erland and Taylor, 2002; Grogan et al., 2000; Jonsson et al., 1999; Peter et al., 2001). One factor contributing to high diversity of ECM communities is niche differentiation regarding different soil substrates (Dickie et al., 2002; Fransson et al., 2000), although its impact on nutrient and water uptake has still not been elucidated.

Differences in the macroscopic features of ectomycorrhizas (color, structure of mantle surface) also revealed differences in the investigated parameters both within and between the stands, which points to variations in the community structure of ECM fungi. The differences in mean thickness of fungal mantle between different stands indicate site-induced variability in fungal symbionts as well. Among the anatomical parameters of short roots, thickness of the mantle had the highest variability (Table 2, Figure 1), which most probably is due to the high diversity of fungal symbionts. Significantly thicker mean fungal mantle in Putkaste is the result of a larger share of black ectomycorrhizas, which as a rule had a thicker mantle compared to others. Thus, the share of different fungal species forming ectomycorrhizas in Norway spruce seems to be an important biotic soil characteristic, which affects both short root structure, in absolute and relative scales, and, most probably, functioning. Our estimates of the share of fungal mantle in the volume of ECM in Norway spruce (from 18 to 28%; Table 3) are close to the results obtained for Scots pine seedlings by Hobbie and Colpaert (2003), where the proportion of fungal tissues in root biomass, estimated by ^{15}N budgeting, ranged from 12 to 22%. Concerning carbon cycling in forest ecosystems, the measurement of carbon cost in ectomycorrhizas is very important. The proportion of mantle in the volume of the ectomycorrhiza provides a rough estimate of the share of the fungal symbiont in mycorrhizal biomass and turnover.

For ectomycorrhizas, the main zone of exchange of mineral and organic nutrients between host and fungal symbiont is the interface between the apoplast and the Hartig net. Bücking and Heyser (2001) noticed that cortical cell nuclei showed a high carbohydrate sink capacity, indicating increased metabolic activity in cortical cells. A larger cortex might provide a larger store for minerals, proteins or lipids (Luxova, 1992). It has also been suggested that the cortex is important for the recycling of phosporus (Robinson, 1990). The number of cell rows (4–6) in the cortex did not vary between our stands, thus, the thickness of the cortex depended mainly on cell size. Our result is in concordance with that of Eissenstat and Achor (1999), who found that larger root diameter among first order roots was caused by larger rather than by more numerous cells in the cortex. The significant positive correlation between site quality class, on one hand, and thickness of the cortex, as well as diameter of stele and root, on the other, indicate the positive impact of site fertility on the dimensions of root tissues of spruce ectomycorrhizas.

According to our earlier results for Norway spruce ectomycorrhizas, SRA is bigger and RTD smaller in sites of higher fertility (Ostonen et al., 1999). Wahl and Ryser (2000) measured root tissue mass density (RTD in present paper) in herbaceous plants and came to a similar conclusion, that low RTD characterised plants of productive habitats, and a high RTD was typical in plants growing in unproductive environments.

RTD, a root characteristic highly sensitive to site and soil conditions and a measure of root functional status (Ostonen et al., 1999), is still not in wide use. The term 'root density' has been taken into use meaning 'rooting density', but already in Sutton and Tinus (1983) different meanings for the term were given, i.e. we cannot use 'root density' analogously to 'wood density' (kg m^{-3}). Hence, there is a need to agree on a new term. In earlier publications 'root density' (kg m^{-3}) for *Picea abies* (Ostonen et al., 1999) or 'tissue mass density in roots' (mg mm^{-3}) for grasses (Wahl and Ryser, 2000) were, for example, used. We support the term 'root tissue density' (RTD).

The sensitivity of stele radius to stand differences was smaller than for other investigated anatomical

variables. Also, Enstone et al. (2001) noticed in the case of *Pinus taeda* that the stele dimensions remained approximately constant. D_{stele} was larger only in the highly-productive Roela spruce stand, where the mean D_{root} was the highest as well. It is reasonable to assume that if the root cortex increases in more fertile habitats due to bigger cortex cells, the size of the stele is increasing as well. The proportion of the stele in root CSA did not vary in different stands (Table 3), and it can be concluded that the parameter is inherent to spruce. Already Wilcox (1971) emphasized that fungal infection does not alter the basic structure of the stele.

The mean CSA percentages of the cortex and stele (fungal mantle excluded) were extremely stable (Table 3); no differences between stands were revealed unless there were big differences in soil parameters and the site quality class varied between IV–V and I[a] (Table 1). The stele CSA in total root CSA in herbaceous plants varied from 8.6 to 25.7% (Wahl and Ryser, 2000), and, as calculated on basis of the results of Eissenstat and Achor (1999), from 12 to 18% for fibrous roots of citrus rootstocks. Thus, from the functional aspect, neither the cortex nor the stele (including incorporated vascular elements) limited the physiological processes in ectomycorrhizas of Norway spruce in relation to quality of the soil and site conditions.

Previous results from the considered study sites showed that soil conditions had a significant impact on ecomorphology of short roots of Norway spruce; the most important morphological parameters related to root functional status were specific root area (absorbing area of dry weight unit), root tissue density (dry weight per water-saturated volume), and diameter of short roots; the SRA was larger, RTD was lower and the mean diameter tended to increase in highly productive spruce stands (Ostonen et al., 1999). Considering the impact of abiotic and biotic site conditions on the pattern of variability of anatomical parameters expressed in the share of mantle, cortex and stele in Norway spruce ectomycorrhizas, the effect of fungal symbiont and site fertility was revealed. T_{cortex} increased with site fertility from Kuusnõmme to Roela (Figure 1). Accordingly to our results, it can be concluded that irrespectively of soil and site conditions, including the influence of fungal symbionts, the proportion of cortex to stele, 4:1 on CSA basis, is inherent to spruce. Further investigation is required to ascertain whether a stable proportion of cortex to stele in root CSA is inherent also to other plant species.

Acknowledgements

This study was supported by Estonian Science Foundation grant No 2487 and No 4895 and by the Swedish Institute. We thank Hans Persson for valuable discussions and excellent working facilities and Kerstin Ahlström from the Swedish University of Agricultural Sciences for kindly introducing her methodology and for thorough technical advice for working with microscope AXIOPHOT. We thank Mr Ilmar Part for revising the English text and two anonymous referees for constructive comments.

References

Agerer R 1987–1997 Colour Atlas of Ectomycorrhizae. 1st–8th edn. Einhorn-Verlag, Swäbisch Gmünd, Germany.

Brandes B, Godbold D L, Kuhn A J and Jentschke G 1998 Nitrogen and phosphorus acquisition by the mycelium of the ecto-mycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. New Phytol. 140, 735–743.

Bücking H and Heyser W 2001 Microautoradiographic localization of phosphate and carbohydrates in mycorrhizal roots of *Populus tremula* × *Populus alba* and the implications for transfer processes in ectomycorrhizal associations. Tree Physiol. 21, 101–107.

Comas L H, Bouma T J and Eissenstat D M 2002 Linking root traits to potential growth rate in six temperate tree species. Oecologia 132, 34–43.

Dahlberg A 2001 Community ecology of ectomycorrhizal fungi: An advancing interdisciplinary field. New Phtyol. 150, 555–562.

Dickie I A, Xu B and Koide R 2002 Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T – RFLP analysis. New Phytol. 156, 527–535.

Egli S, Amiet R, Zollinger M and Schneider B 1993 Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. Trees 7, 123–129.

Eissenstat D M and Achor D S 1999 Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytol. 141, 309–321.

Ekblad A, Wallander H, Carlsson R and Huss-Danell K 1995 Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. New Phytol. 131, 443–451.

Enstone D E, Peterson C A and Hallgren S W 2001 Anatomy seedling tap roots of loblolly pine (*Pinus taeda* L.). Trees 15, 98–111.

Erland S and Taylor A F S 2002 Diversity of Ecto-mycorrhizal Fungal Communities in Relation to the Abiotic Environment. *In* Mycorrhizal Ecology. Ecological Studies, Vol. 157. Eds. M G A van der Heijden and I Sanders. pp. 163–200. Springer-Verlag, Berlin Heidelberg.

Fitter A H 1985 Functional significance of root morphology and root system architecture. *In* Ecological Interactions in Soil. Special Publication of the British Ecological Society, No 4. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp. 87–106. Blackwell Scientific, Oxford, UK.

Ford E D and Deans J D 1977 Growth of a Sitka spruce plantation: Spatial distribution and seasonal fluctuations of lengths, weights and carbohydrate concentrations of fine roots. Plant Soil 47, 463–485.

Fransson P M A, Taylor A F S and Finlay R D 2000 Effects of continuous optimal fertilization on belowground ectomycorrhizal community structure in a Norway spruce forest. Tree Physiol. 20, 599–606.

Grogan P, Baar J and Bruns T D 2000 Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. J. Ecol. 88, 1–13.

Hobbie E A and Colpaert J V 2003 Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol. 157, 115–126.

Horton T R and Bruns T D 2001 The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. Mol. Ecol. 10, 1855–1871.

Högberg P, Nordgren A, Buchmann N, Taylor A F S, Ekblad A, Högberg m N, Nyberg G, Ottosson-Löfvenius M and Read D J 2001 Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.

Jonsson L, Dahlberg A, Nilsson M-C, Kåren O and Zackrisson O 1999 Continuity of ectomycorrhizal fungi in self-regenerating boreal forests of *Pinus sylvestris*: Comparative analysis of diversity of mycobionts of seedlings and old trees. New Phytol. 142, 151–162.

Kåren O 1997 Effects of Air Pollution and Forest Regeneration Methods on the Community Structure of Ectomycorrhizal Fungi. Ph.D. Thesis,Swedish University of Agricultural Sciences.

Leake J R and Read D J 1997 Mycorrhizal fungi in terrestrial ecosystems. *In* The Mycota IV. Experimental and Microbial Relationships. Eds. D Wicklow and B Soderström. pp. 281–301. Springer, Berlin Heidelberg New York.

Lindahl B, Stenhild J, Olsson S and Finlay R 1999 Translocation of ^{32}P between interacting mycelia of a wood decomposing fungus and ectomycorrhizal fungi in microcosm systems. New Phytol. 144, 183–193.

Lindahl B O, Taylor A F S and Finlay R D 2002 Defining nutritional constraints on carbon cycling in boreal forests – Towards a less 'phytocentric' perspective. Plant Soil 242, 123–135.

Löhmus K and Ivask M 1995 Decomposition and nitrogen dynamics of fine roots of Norway spruce (*Picea abies* (L.) Karst.) at different sites. Plant Soil 168–169.

Löhmus K and Lasn R 1990 Spruce and pine root structures and chemical characteristics in moderate acid soils. Air Poll. Res. Rep. 32, 74–78.

Löhmus K, Oja T and Lasn R 1989 Specific root area: A soil characteristic. Plant Soil 119, 245–249.

Luxova M 1992 Root structure. Primary cortex. *In* Physiology of Plant Root System. Eds. J Kolek and V Kozinka. pp. 52–59. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Majdi H 1994 Effects of nutrient applications on fine–root dynamics and root/rhizosphere chemistry in a Norway spruce stand. Ph.D. Thesis, Swedish University of Agricultural Sciences. Report 71. 102 pp.

Niinemets Ü and Kull O 1998 Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: implications for foliage morphological plasticity. Tree Physiol. 18, 467–479.

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

Perez-Moreno J and Read D J 2000 Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. 145, 301–309.

Persson H, Ahlström K and Clemensson-Lindell A 1998 Nitrogen addition and removal at Gårdsjön – Effects on fine-root growth and fine-root chemistry. For. Ecol. Manag. 101, 199–205.

Peter M, Ayer F and Egli S 2001 Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytol. 149, 311–325.

Pregitzer K S, DeForest J L, Burton A J, Allen M F, Ruess R W and Hendrick R L 2002 Fine root architecture of nine North American trees. Ecol. Monogr. 72, 93–309.

Read D J and Perez-Moreno J 2003 Mycorrhizas and nutrient cycling in ecosystems – A journey towards relevance? New Phytol. 157, 475–492.

Robinson D 1990 Phosphorus availability and cortical senescence in cereal roots. J. Theor. Biol. 145, 257–265.

Rousseau J V D and Reid C P P 1989 Measurement of Carbon Cost in Ectomycorrhizae. *In* Applications of Continuous and Steady-State Methods to Root Biology. Eds. J G Torrey and L J Winship. pp. 183–196. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sarand I, Timonen S, NurmiahoLassila E L, Koivula T, Haahtela K, Romantschuk M and Sen R 1998 Microbial biofilms and catabolic plasmid harbouring degradative fluorescent pseudomonads in Scot pine mycorrizospheres developed on petroleum contaminated soil. FEMS Microbiol. Ecol. 27, 115–126.

Simard W S, Jones M D and Durall D M 2002 Carbon and Nutrient Fluxes Within and Between Mycorrhizal Plants. *In* Mycorrhizal Ecology. Ecological Studies, Vol. 157. Eds. M G A van der Heijden and I Sanders. pp. 34–74. Springer-Verlag, Berlin Heidelberg.

Sutton R F and Tinus R W 1983 Root and root system terminology. For. Sci. Monogr. 24, 137 pp.

Söderström B E and Bååth E 1978 Soil microfungi in three Swedish coniferous forests. Hol. Ecol. 1, 62–72.

Taylor A F S 2002 Fungal diversity in ectomycorrhizal communities: Sampling effort and species detection. Plant Soil 244, 19–28.

Wahl S and Ryser P 2000 Root tissue structure is linked to ecological strategies of grasses. New Phytol. 148, 459–471.

Wilcox H E 1971 Morphology of Ectendomycorrhizae in *Pinus resinosa*. *In* Mycorrhizae. Ed. E Hacskaylo. pp. 54–68. Proceedings of the first North American conference on mycorrhizae, April 1969. Misc. Publication 1189 U. S. Department of Agriculture, Forest Service, Washington.

Section editor: J.W.G. Cairney

VI

Granhall U, Lõhmus K, Püttsepp Ü, Ostonen I. 2003. **Mycorrhizae in *Alnus incana*.** In: Eds. Ü. Mander and K. Lõhmus. Riparian alder forests: Their importance as buffer zones and bioenergy sources. Kluwer Academic Publishers, Dordrecht. (Accepted)

Chapter 3

MYCORRHIZAE IN *ALNUS INCANA*

Ulf Granhall[1], Krista Lõhmus[2], Ülle Püttsepp[3,4], Ivika Ostonen[2,4]
[1]*Department of Microbiology, Swedish University of Agricultural Sciences*
Box 7025, SE-75007 Uppsala, Sweden
E-mail: Ulf.Granhall@mikrob.slu.se
[2]*Institute of Geography, University of Tartu*
Vanemuise 46, 51014 Tartu, Estonia
[3]*Institute of Zoology and Botany, Estonian Agricultural University*
Riia 181, 51014 Tartu, Estonia
[4]*Institute of Botany and Ecology, University of Tartu*
Lai 40, 51005 Tartu, Estonia

INTRODUCTION

The genus *Alnus* may host three different symbiotic partners: the nitrogen-fixing actino-mycete *Frankia*, and ecto- and endomycorrhizas. Tetrapartite symbiosis is considered to be the most beneficial for the growth of e.g. *A. incana* seedlings (Chatarpaul et al., 1989), since both mycorrhizal types dramatically increase phosphorus uptake and thereby stimulate nitrogen fixation and/or nodulation by *Frankia.* Tetrapartite association in *A. incana* is also reported by Arveby & Granhall (1998), in *A. glutinosa* by Hall et al. (1979) and Gardner et al. (1984), and in *A. rubra* and *A. sinuata* by Rose (1980).

The arbuscular mycorrhizal fungi (AM/VAM/endomycorrhizae) are considered to be little host-specific and three of the four major genera (*Acaulospora, Gigaspora* and *Glomus*) have been found associated with different alder species, including *A. incana* (Rose, 1980).

In contrast to the AM fungi, ectomycorrhizal fungi that infect alders seem to be quite host-specific (Mejstrik & Benecke, 1969; Molina, 1979, Molina 1981; Murphy & Miller, 1994; Pritsch, 1996; Pritsch et al., 1997; Arveby & Granhall, 1998). Reported exceptions are *Telephora terrestris* (Miller et al., 1992) and a few other broad-range fungi e.g. *Cenococcum geophilum,*

Hebeloma spp., and *Laccaria laccata* (Arveby & Granhall, 1998). The specificity is thought to be intrageneric (Molina, 1979). Specific alder fungi include representatives of genera like: *Alnirhiza, Alpova, Amanita, Cortinarius, Gyrodon, Lactarius, Paxillus*, and *Russula.* In natural grey alder (*A. incana*) stands in Sweden, Arveby & Granhall (1998) found 30 species, and Pritsch et al. (1997) reported 16 different ectomycorrhizal types in black alder (*A. glutinosa)* forests in Germany.

A succession from AM fungi to broad-range early-stage ectomycorrhizal fungi, to specific late-stage ones has been found in *A. incana* (Arveby & Granhall, 1998). Although some of these steps are quite similar in *A. glutinosa* (Fraga-Beddiar, 1987) and *Betula* spp. (Deacon et al., 1983; Last et al., 1987), species differences among alders obviously also occur, since such distinct succession stages are not seen in e.g. *A. rubra* (Miller et al., 1992) or *A. crispa* and *A. rugosa* (Fortin & Carlisle, 1984).

The occurrence, succession and significance of mycorrhizas in alders, with particular emphasis on grey alder (*Alnus incana* (L.) Moench.) will be surveyed and discussed.

1. MATERIAL AND METHODS

1.1 Mycorrhizal survey

Root sampling was carried out in planted and natural grey alder sites in Sweden and Estonia. Different stand ages, soil textures, pH, and fertilization regimes were represented (Table 1).

Root samples from planted Swedish grey alder stands were collected with soil augers close to (0-1 m) single trees and down to 40 cm. Storing, washing, fixation, and staining of samples, as well as scanning procedures for AM and ectomycorrhizal fungi (ECM) for the Swedish samples are fully described or referred to in Arveby & Granhall (1998). The method of Phillips & Hayman (1970), with certain modifications, was used as the principal technique for mycorrhizal scannings (both AM and ECM) and the method of Kähr & Arveby (1986) for verification and description of ECM infections.

The Estonian samples from natural grey alder stands (*Aegopodium* site type) were treated as follows: roots for mycorrhizal detection were collected from *A. incana* stands in the Koka, Ilmatsalu, Porijõgi and Viiratsi sites (southern Estonia) during 1996 to 2000. Stand ages were 18, 6, 14 and 40 years, respectively (Table 1). Ten to twenty samples from each site were taken randomly by spading the soil surface layer of 20 cm in depth (Koka

and Ilmatsalu) or using a soil corer (Ø 147 mm). From each set of 10 samples, fine roots, <2 mm in diameter, were collected and gathered to a composite sample. Three to four sub-samples were taken from each composite sample for mycorrhizal analysis. Root sub-samples were stored in 50% ethanol until processing.

A 6-year-old experimental plantation on an abandoned field at Holvandi was also investigated (Uri et al., 2002) by taking twenty soil cores (Ø 48 mm) down to a 40 cm depth in November 1998. Soil samples were divided into 10 cm layers and kept in a refrigerator at 5 °C until processing.

In the Holvandi plantation, in-growth cores were also collected in November 2000; the in-growth cores (Ø 40 mm, mesh size 7 mm) were installed in November 1999 down to a 30 cm depth. The cores were in this case divided into the following depth layers: 0-5, 5-10, 10-15, 15-20, and 20-30 cm. Further processing was as described above.

To reveal the presence of both AM and ectomycorrhizal fungi in the roots the procedure by Phillips & Hayman (1970), as modified by Koske & Gemma (1989), was applied. In our case heating at 90°C in 2.5 % KOH for 25 minutes was used for clearing, and soaking in 1 % HCl for 24 hours for acidification. Root specimens were also frozen and cut in a microtome cryostat (Microm HM 500 OM). The embedding medium for frozen specimens was TISSUE-TEC (O.C.T. 4583 compound, MILES-USA). Cross- or transverse sections (5 µm thick) were stained in Methylene Blue and examined by light microscope AXIOPHOT, magnification 200-800 x.

Observations of characteristics that easily change at preserving, such as colour, were done on fresh material in all cases.

Results from tests on inoculum content of some of the Swedish soils (as reported by Arveby & Granhall, 1998) and other published infection and synthesis experiments with grey alder will be commented upon in combination with observations from the field, i.e the above mycorrhizal survey will be complemented with nodulation data.

1.2 Influence of mycorrhizae on growth of *A. incana*.

The overall picture of mycorrhizal effects on grey alder will be discussed with respect to observations under controlled experimental conditions and those made in the field, in both planted and natural stands of different ages. Material and methods are fully described in cited original papers.

2. RESULTS AND DISCUSSION

2.1 Mycorrhizal survey

The occurrence of arbuscular and ectomycorrhizal fungi, and actinorhiza, in eight planted Swedish and five Estonian (one planted and four natural) grey alder stands is presented in Table 1.

As seen in Table 1, location, soil texture, pH, fertilization regime, and stand age had no apparent influence on the overall appearance of ECM, except for giving weak formation on peat soils (cf. below).

AM-infection, however, was negatively influenced by stand age (more than 5-year-old trees seldom had AM and then always in combination with ECM). In this case, the soil type dominated by peat (ombrotrophic Sphagnum peat) was also different from the others in the sense that AM infection was totally absent (cf. below).

The inoculum content, *i.e.* time and frequency of infection, of four different Swedish soils, two of which (Ultuna, Studsvik) are represented in Table 1, has been described by Arveby and Granhall (1998). Also the nodulation pattern was given, to demonstrate that *Frankia* infection, if occurring, always preceded mycorrhizal infection. The result was in agreement with early season collections of saplings which were occasionally nodulated but non-mycorrhizal. The reverse situation was not found. No nodule formation was observed and no mycorrhizas were formed in the peat soil even after 12 weeks.

This and other experiments (Granhall et al., 1983; Huss-Danell & Frej, 1986; Arveby & Huss-Danell, 1988) show that previously uncultivated peat soils do not harbour the necessary endophytes for establishment of grey alder plantations, *i.e.* inoculation with *Frankia* is absolutely necessary, and, if AM fungi are absent, P-fertilization is a must during establishment (the plants died in absence of added P; Granhall, U. unpubl.). After a year or two, at least weak ectomycorrhiza was formed in all studied peat soils (cf. Table 1; and Arveby & Granhall, 1998).

In contrast to the uncultivated peat soil an alder site soil was found to be the most suitable for the development of the grey alder seedlings. Here, nodules were formed after 2 to 3 weeks and AM-infection was established soon after. ECM-infection, however, was not noted until the last harvest, after 12 weeks, when a few *Cenococcum geophilum* (broad-range) ectomycorrhizas were found.

In the other two mineral soils (Studsvik, Ultuna cf. Table 1) studied (no previous alder plants) nodulation was confirmed after 3-4 weeks and AM-

infection progressed slowly thereafter. No signs of ECM-infection were noted after 12 weeks in these soils, although it was easily detected in the field (cf. Table 1).

In vitro inoculation of nodulated grey alder seedlings with *Glomus mossae* (Nicol. & Gerd.) Gerdemann & Trappe results in AM-infection (Arveby & Granhall, 1998). Simultaneous syntheses with a number of ECM isolates, however, indicate that the grey alder plant host is resistant to ECM infection in the sapling stage, except for some pseudomycorrhizal sheaths being formed by a few broad-range early-stage ECM-fungi like *Paxillus involutus*. This is in keeping with observations in *A. glutinosa* (Fraga-Beddiar, 1987) but in contrast to those in *A. rubra* (Miller et al., 1992), *A. crispa* and *A. rugosa* (Goudbout & Fortin, 1983).

Field observations of planted grey alder saplings in mineral soils occasionally revealed ECM-infections but only in late seasonal samplings, i.e. 5-6 months after planting, and always combined with AM-infection (Arveby & Granhall, 1998).

In trees over one year old, AM-infections occur irregularly with few arbuscules and always together with ECM (cf. Table 1). Single roots may harbour both mycorrhizal types simultaneously. In such cases ectomycorrhizal mantles are generally thin indicating competition between fungi.

In older natural stands ECM is totally dominating and usually already obvious at macroscopic observation. The mantle (Figure 1) may be smooth or rough. The mantle region is extended 2-8 mm from the root apex in mature grey alder trees. The surface of the mantle has a net-like structure of branched and lobed, densily adjoining hyphae. Loose hyphae extending from the mantle occurred abundantly, forming an extramatrical mycelium. In some (Estonian) samples rhizomorphs, i.e. multihyphal linear aggregates, were seen. ECM structures were observed in 89 % and 67 % of subsamples in Porijõgi and Viiratsi study-sites, respectively (Estonia). The figure refers to the extent of ECM colonization across the whole fraction of the fine roots studied and not only the root tip, the most extensively colonised region. Aggregates of ramified ectomycorrhizal root-tips (Figure 2), 20-50 mm in diameter occurred in both the Porijõgi and Viiratsi sites.

Summarizing the experience from all Swedish and Estonian sites it can be concluded that a mantle and a Hartig net were, except for peat sites, always present in root samples of mature grey alders. The thickness of the mantle varied. The Hartig net was epidermal and did not penetrate deeper. Radial elongation of infected epidermal cells, a common feature of angiosperm sheathing mycorrhizas, was not observed and confirms earlier results that it seldom seem to occur in alder mycorrhizae (Masui, 1926; Neal

et al., 1968; Molina, 1979; Godbout & Fortin, 1983; Miller et al., 1992; Arveby & Granhall, 1998).

The general visual appearance of the grey alder ectomycorrhizas varied with the type and age of stands. In planted stands (Swedish stands up to 6 years of age) swellings of mycorrhizal root tips and development of more prominent mycorrhizal mantles seldom occurred. Colouration varied mainly between translucent/whitish and deep brown. Mantle surfaces were mostly smooth and often surrounded by loose hyphae.

In natural stands and old planted stands, from both countries, the mycorrhizas generally had slightly enlarged second or third order pinnate ramified root-tips with mantles ranging from smooth to rough with loose hyphae or totally inwoven by extramatrical mycelium. Whitish mantles dominated in Swedish specimens, but red, brown, and yellow types also occurred. Estonian specimens were predominantly whitish in the planted study site on abandoned field, the natural sites being dominated by light-brown, purple-brown and deep-brown ectomycorrhizas. However, studies on the ECM community composition are needed to reveal any possible regional differences.

2.2 Influence of mycorrhizae on growth of *A. incana*

Except for general observations under controlled laboratory conditions that both AM and ECM fungi constitute carbon sinks for the grey alder host plant (saplings), as in all other mycorrhizal symbioses, and thereby cause some energy (biomass) losses (Arveby & Granhall, 1998; Ekblad & Huss-Danell, 1994), there are, as far as we know, no reports on the long term effects of mycorrhizae on the growth performance of grey alder. Our own field observations are that grey alder may grow quite well, devoid of AM and at least specific ECM (Arveby & Granhall, 1998), provided that nodulation is satisfactory and that P is available in the soil, since nodulated non-mycorrhizal plants that were provided with PO_4-P thrived well (Rytter et al., 1989; 1990; 1991) on the ombrotrophic peat bog Stormuren (cf. Table 1), in contrast to P-unfertilized ones that did not survive (data not shown). Other field observations from the same site were, however, that nodulated plants supplied with soil containing AM and ECM fungi infectious to grey alder showed increased survival rate (92% vs. 84%, 2-3 years after planting).

As phosphorus (P) is the most limiting nutrient factor for most nitrogen fixing plants (Sprent, 1979), ECM should be essential for the optimization of the nitrogen fixation process in grey alder also (cf. Chatarpaul et al., 1989). Significant growth responses to P-fertilization have been noted in planted *A. incana* stands both in Finland (Hytönen et al., 1995) and Sweden (Granhall, U., unpubl.). For example, mean heights of plants in 3-year-old stands at

Studsvik (heavy clay soil, cf. Table 1) were 3.50 ± 0.09 (SE) m and 2.42 ± 0.07 (SE) m, with and without P-amendments, respectively. The concentrations of N and P in leaves were significantly correlated ($r = 0.82$, $p < 0.01$) taking both treatments into account. Interestingly Uri (2002) noted that, when comparing the content of available phosphorus in the soil at the time of plantation of a grey alder stand and at the second growing season, it had more than redoubled in the upper soil layers. This could be an effect of the myorrhizas and/or the root excretion of phosphatases (Giardina et al., 1995).

Other observations suggest that the below-ground carbon flow of alders is directed primarily to the *Frankia* symbiont, since a restricted flow of carbon to the ECM is most likely owing to the limited interface (superficial Hartig net) for nutrient transfer (N, P etc.) between the ectomycorrhizae and the plant host. A striking feature is also that relatively small fruit bodies are typically formed by several specific "alder fungi" in natural stands of *A. incana* compared to those formed by the same fungal genera with other host plants (Arveby & Granhall, 1998).

In the Estonian samples, ECM frequency was lower (data not shown) in the planted stand (Holvandi) compared to the natural stands, although transplants of natural origin were used. Whether this has to do with the absence of certain ECM fungi at this particular site or stand age (6 years) is not known at present. In planted stands in Sweden no fruit bodies of ECM fungi were found in stands less than five years of age. All investigated trees in natural stands older than 5 years, both in Sweden and Estonia, were nodulated and had a high frequency of ECM, and generally lacked AM (cf. Table 1; Arveby & Granhall 1998).

3. CONCLUSIONS

Ectotrophic mycorrhiza (ECM) was found always to be present and arbuscular mycorrhiza (AM) mostly absent in grey alder stands more than five years old. ECM infections seem often to be weak for at least five years in soils previously uncultivated with alders.

The balance between early and persistent *Frankia* infection, immediately followed by AM fungi, succeeded by broad-range early-stage ECM and followed by specific late-stage ECM species in grey alder (Arveby & Granhall, 1998) is truly an intricate and versatile one that seem to partition the carbon demand temporally between the symbiotic partners in an optimal way under most natural conditions, provided that all partners are present.

Whether the introduction of selected mycorrhizal partners would contribute to the productivity of planted alders in areas where alders have

not been grown previously has unfortunately not been investigated. Meanwhile, checks on the "mycorrhizal status" of planted grey alders in soils with low available P-levels are recommended in order to counteract P limitations for growth, either by P-fertilization (short-run) or possibly mycorrhizal-introduction (long run). The apparent resistance of grey alders to ECM at seedling/sapling stages, however, makes it difficult to induce symbioses with these fungi in plant nurseries, for example. AM fungi and *Telephora terrestris* (early-stage, broad-range ECM fungus that "infests" most forest nurseries) could be candidates though, which later may be "naturally" replaced by more specific ECM on site. As the famous mycologist Donald Marx put it: "Any mycorrhiza is better than no mycorrhiza (pers. comm.)."

REFERENCES

Arveby, A. S., Granhall, U., 1998. Occurrence and succession of mycorrhizas in *Alnus incana*. Swedish J. agric. Res, 28, 117-127.

Arveby, A. S., Huss-Danell, K., 1988. Presence and dispersal of infective *Frankia* in peat and meadow soils in Sweden. Biol. Fertil. Soils, 6, 39-44.

Chatarpaul, L., Chakravarty, P., Subramaniam, P., 1989. Studies in tetrapartite symbioses I. Role of ecto- and endomycorrhizal fungi and *Frankia* on the growth performance of *Alnus incana*. Plant and Soil, 118, 145-150.

Deacon, J. W., Donaldson, S. J., Last, F. T., 1983. Sequences and interactions of mycorrhizal fungi on birch. Plant and Soil, 71, 257-262.

Ekblad, A., Huss-Danell, K. 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. New Phytologist, 131, 453-459.

Fortin, J. A., Carlisle, A., 1984. The Use of Root Symbiosis in Intensive Forestry. IEA/FE Programme Group 'B'- ENFOR CFS, Report 1984:4.

Fraga-Beddiar, A., 1987. Interaction entre les symbiotes mycorhiziens et les symbiotes fixateurs d'azote chez l'Aulne glutineux (*Alnus glutinosa* (L.) Gaertn.). Thesè de Docteur de 3e Cycle. Univ. de Nancy, Lab. De Microbiol. For. I.J.R.A., Nancy.

Gardner, I. C., Clelland, D. M., Scott, A., 1984. Mycorrhizal improvement in non-leguminous nitrogen fixing associations with particular reference to *Hippophaë rhamnoides* L. Plant and Soil, 78, 189-200.

Giardina, C. P., Huffmann, S., Binkley, D., Caldwell, B. A., 1995. Alders increase soil phosphorus availability in a Douglas Fir plantation. Can. J. For. Res., 25, 1652-1657.

Goudbout, C., Fortin, J. A. 1983. Morphological features of synthesized ectomycorrhizae of *Alnus crispa* and *A. rugosa*. New Phytologist, 94, 249-252.

Granhall, U., Ericsson, T., Clarholm, M., 1983. Dinitrogen fixation and nodulation by *Frankia* in *Alnus incana* as affected by inorganic nitrogen in pot experiments with peat. Can. J. Bot., 61, 2956-2963.

Hall, R. B., McNabb, H. S. Jr, Maynard, C. A., Green, T. L., 1979. Toward development of optimal *Alnus glutinosa* symbioses. Bot. Gaz, 140 (Suppl.), 120-126.

Huss-Danell, K., Frej, A. K., 1986. Distribution of *Frankia* in soils from forest and afforestation sites in northern Sweden. Plant Soil, 90, 407-418.

Hytönen, J., Saarsalmi, A., Rossi, P., 1995. Biomass production and nutrient consumption of short-rotation plantations. Silva Fennica, 29 ,2, 117-139.

Koske, R. E., Gemma, J. N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 4, 486-488.

Kähr, M., Arveby, A. S., 1986. A method for establishing ectomycorrhiza on conifer seedlings in steady-state conditions of nutrition. Physiologia Plantarum, 67, 333-339.

Last, F. T., Dighton, J., Mason, P. A., 1987. Successions of sheathing mycorrhizal fungi. Trends in Ecology and Evolution, 2, 157-161.

Masui, K., 1926. A study of the ectotrophic mycorrhiza of Alnus. Memoirs of the College of Science, Kyoto Imperial University, Series B, Volume II: 4, article 10, 190-210.

Mejstrik, V., Benecke, U., 1969. The ectotrophic mycorrhizas of *Alnus viridis* (Chaix) D. C. and their significance in respect to phosphorus uptake. New Phytologist, 68, 141-149.

Miller, S. L., Koo, S. D., Molina, R., 1992. Early colonization of red alder and Douglas fir by ectomycorrhizal fungi and *Frankia* in soils from the Oregon coast range. Mycorrhiza, 2, 53-61.

Molina, R., 1979. Pure culture synthesis and host specificity of red alder mycorrhizae. Canadian Journal of Botany, 57, 1223-1228.

Molina, R., 1981. Ectomycorrhizal specificity in the genus *Alnus*. Canadian Journal of Botany, 59, 325-334.

Murphy, J. F., Miller, O. K. Jr., 1994. Mycorrhizal syntheses with *Alnus serrulata* (Ait.) Willd. Castanea, 59, 156-166.

Neal, J.L., Trappe, J. M., Lu, K.C., Bollen, W. B., 1968. Some ectotrophic mycorrhizas of Alnus rubra. In: Biology of Alder (Eds. J.M. Trappe, J.F.Franklin, R.F. Tarrant, G..M. Hansen) 179-184. Northwest Science Association, 40th Annual Meeting, Symposium Proceedings 1967.

Phillips, J. M., Hayman, D. S., 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55, 158-161.

Pritsch, K., 1996. Untersuchungen zur Diversität und Ökologie von Mykorrhizen der Schwarzerle (*Alnus glutinosa* (L.) Gaertn.). PhD Thesis. Universitet Tübingen.

Pritsch, K., Boyle, H., Munch, J. C., Buscot, F., 1997. Characterization and identification of black alder ectomycorrhizas by PCR/RFLP analyses of the rDNA internal transcribed spacer (ITS). New Phytologist, 137, 357-369.

Rose, S. L., 1980. Mycorrhizal associations of some actinomycete nodulated nitrogen-fixing plants. Canadian Journal of Botany, 58, 1449-1454.

Rytter, L., Slapokas, T., Granhall, U., 1989. Woody biomass and litter production of fertilized grey alder plantations on a low-humified peat bog. For. Ecol. Manage., 28, 161-176.

Rytter, L., Granhall, U., Arveby, A. S., 1990. Experiences of grey alder plantations on a peat bog. In: Fast Growing Trees and Nitrogen Fixing Trees, Int. Conf. Marburg, F.R.G., 8-12 Oct 1989, eds. Werner, D., Müller, P., 112-114. Stuttgart, Gustav Fischer Verlag.

Rytter, L., Arveby, A. S., Granhall, U., 1991. Dinitrogen (C_2H_2) fixation in relation to nitrogen fertilization of grey alder plantations on a peat bog. Biol. Fertil. Soils, 10, 233-240.

Sprent, J. I., 1979. The Biology of Nitrogen Fixing Organisms. London, McGraw-Hill Company.

Uri, V., Tullus, H. and Lõhmus, K. 2002. Biomass production and nutrient accumulation in short-rotation grey alder (*Alnus incana* (L.) Moench) plantation on abandoned agricultural land. Forest Ecology and Management 161 (1-3), 169-179.

Table 1. Site description of grey alder stands surveyed for mycorrhizal and actinorhizal occurrences by root sampling. AM = arbuscular mycorrhiza, ECM = ectomycorrhiza, NOD = actinorhiza (+ = presence, – = absence). Extended and rearranged data from Arveby & Granhall (1998) for Swedish sites. PL = planted, N = natural.

Location	Soil texture	pH	Fertilization	Stand age (yrs)	AM	ECM	NOD
Stormuren[1], plot G12, PL	peat	6.1	P. ash (K), Ca	1,2	–	(+)	+
Stormuren[1], plot A26-36, PL	peat	5.5	N, P, K, Ca	2–4	–	(+)	+
Finnmossen[1], PL	peat	4.5	N, P, K, Ca	3	–	(+)	+
Stormuren[1], plot G43, PL	peat	4.9	P, K, Ca	4,5	–	(+)	+
Ultuna[1], PL	Clay loam	7.5	P, K	2,3	+	+	+
Studsvik[1], PL	Silty loam	7.4	P, K	4,5	+	+	+
Umeå[2], PL	silt	5.2	–	5	+	+	+
Ekebo[3], PL	loam	6.4	–	27	(+)	+	+
Holvandi[4], PL	loam	6.4	–	6	–	+	+
Ilmatsalu[4], N	loam		–	6	–	+	+
Koka[4], N	loam		–	18	–	+	+
Porijõe[4], N	Sandy loam	6.4	(N, P from overflow)	14	–	+	+
Viiratsi[4], N	Sandy loam		(N, P from overflow)	40	–	+	+

[1] Central Sweden
[2] Northern Sweden
[3] Southern Sweden
[4] Southern Estonia

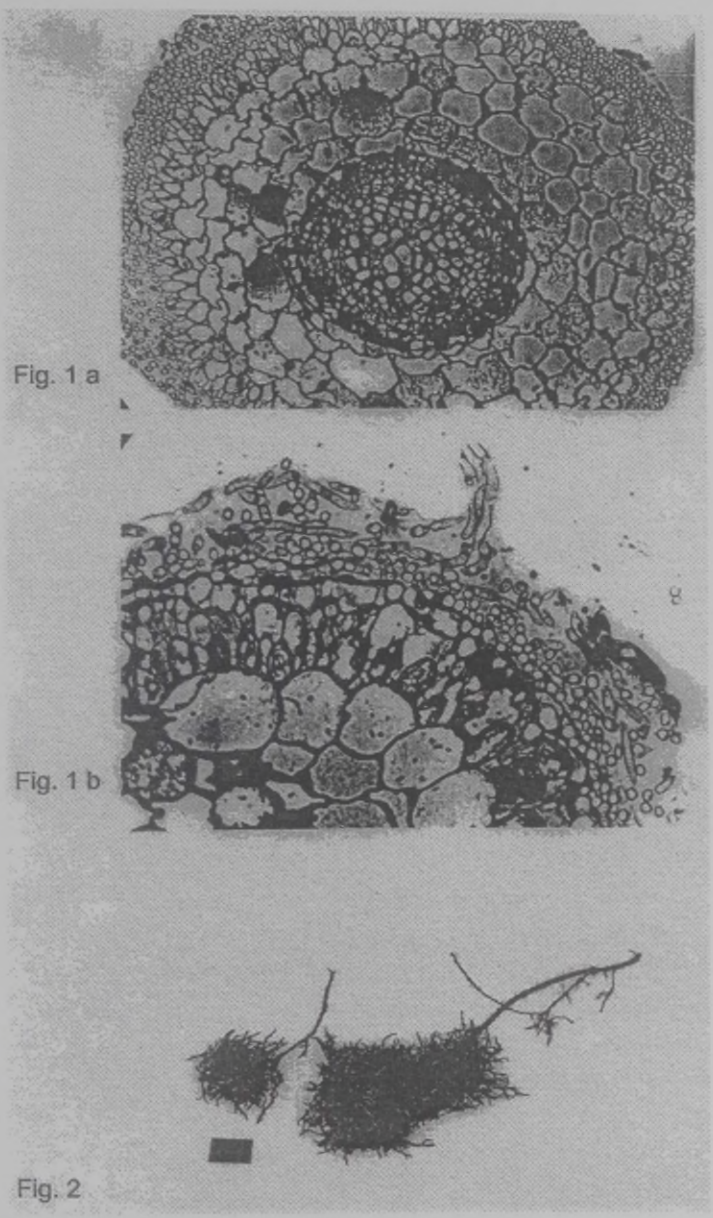

Figure 1. Ectomycorrhizal infection in *Alnus incana* roots. Samples were fixed in 4% glutaraldehyde, embedded in Historesin, and stained in Löffler's methylene blue. Cross section of 3 µm showing rough (1a) and smooth (1b) mantle and epidermal Hartig net.

Figure 2. Aggregated ecomycorrhizas of *Alnus incana* (Viiratsi site). Bar is 1 cm.

VII

Ostonen I, Lõhmus K, Pajuste K.
Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce stand: comparison of soil core and ingrowth core methods. (submitted to Ecosystems)

Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: comparison of soil core and ingrowth core methods

Ivika Ostonen[1, 2], Krista Lõhmus[1] and Katrin Pajuste[1]

[1] Institute of Geography, University of Tartu, Vanemuise 46, 51014, Estonia
[2] Institute of Botany and Ecology, University of Tartu, Lai 40, 51005, Estonia

Abstract

Fine root bio- and necromass, net primary production (NPP) of fine roots and its proportion of the NPP of trees, as well as turnover rate were investigated in a fertile middle-aged Norway spruce (*Picea abies* (L.) Karst) stand by sequential core and ingrowth core methods. The stand's site type is *Oxalis,* the site quality class is I^a and the soil type is Umbric Luvisol (FAO classification). Twenty soil cores (volumetric samples, core diameter 38 mm) were taken monthly during the period June-1996 to November 1996 and in June-1997. Ingrowth cores were collected, fifteen at a time, during the growing seasons from 1997 to 1999, once after one year and three times in the second and third years. Spruce roots from samples collected by both methods were separated into living and dead roots (two diameter classes: <1 mm and 1 mm ≤ d <2 mm). The fine root NPP was calculated according to the decision matrix, and root turnover rate was calculated as annual root production divided by mean fine root biomass.

The mean biomass of fine (<2mm) and finest (<1 mm) roots in ingrowth cores collected in the third year after installation was two times smaller than that in soil cores. The mean fineroot biomass was 1420 ± 170 kg ha^{-1} in soil cores and 700 ± 105 kg ha^{-1} in the third year ingrowth cores. The finest roots formed ca 2/3 of fine root biomass. The fine root NPP estimated by the sequential core method was 2.5 t ha^{-1} yr^{-1} and 1.0 t ha^{-1} yr^{-1} by the ingrowth core method (third year after installation). The fine root turnover rate was 1.8 yr^{-1} for sequential cores and 1.4 yr^{-1} for third-year ingrowth cores. The inverse of the root turnover rate is, in turn, a measure of average root longevity; it was smaller for the finest roots in both cases. In the investigated spruce stand the annual NPP of trees at the age of 40 years is estimated as 21.4 t ha^{-1} yr^{-1}, the share of the belowground part forming 31%. Fine roots accounted for 13% of the NPP, which is a relatively small value compared to the results revealed in most studies.

Introduction

Fine root net primary production constitutes an important, but often unmeasured, part of the carbon budget of forest ecosystems. Direct measurements are problematic in many ways, and the assessment methods are extremely labour-intensive. Various methods, both direct and indirect, have been used to measure fine root biomass and production — most often by sequential coring (Ahlström et al., 1988; Fairley and Alexander, 1985; Helmisaari et al., 2002; Persson, 1978; Yin et al., 1989) or ingrowth cores (Jones et al., 2003; Makkonen and Helmisaari, 1999; Persson, 1983), minirhizotron method (Burton et al., 2000; King et al., 2002; Majdi and Nylund, 1996; Majdi and Kangas, 1997), and indirect methods such as the N budget (Aber et al., 1985; Nadelhoffer et al., 1985). A critical review of the existing root biomass and NPP assessment methods and their advantages and disadvantages was published by Vogt et al. (1998) and some aspects of problems and progress in estimating fine root production were discussed by Nadelhoffer (2000).

However, tremendous controversy exists in the published literature as to which is the best method to determine fine root biomass and NPP. One of the reasons for controversy in estimating fine root production and turnover in forests is that trees have highly variable patterns of allocation of photosynthates to fine roots (varying from 4 – 69 % of total plant carbon annually fixed), which can therefore significantly affect the ecosystem-level processes (Vogt et al., 1996). Carbon is translocated from aboveground part to the root system during root growth and maintenance, and is added to the mineral soil and forest floor carbon pools via hyphae at ectomycorrhizas and rhizodeposition as root litter or root exudates. Microbial communities in soil, rhizosphere and in decomposing root litter are supported by assimilates from trees (Lõhmus and Ivask, 1995; Read, 1997). The estimate of net photosynthates allocated to mycorrhizal fungi can range from 5 to 85 % among different systems (Allen, 1991). Further, there was discussed in the literature how big rate of the C allocated to roots is respired (Högberg et al., 2001, 2002). Hence, more empirical data on the belowground part of forest ecosystem are needed.

There is very important to understand how soil environmental factors, such as nutrient availability, water conditions, temperature etc., affect fine root turnover and influence C allocation strategies at the scale of tree or whole forest ecosystem. Improved nutrient availability sometimes leads to decreases in fine root production (in absolute terms or as a proportion of total stand production) and biomass within forest type (Haynes and Gower, 1995), but evidence exists for both increased (Keyes and Grier,1981; Pregitzer et al., 1993; Vogt et al., 1986) and decreased (Aber et al., 1985; Nadelhoffer et al., 1985, 2000; Pregizer et al., 1995) fine root life spans in more fertile soils. In optimal site conditions, both the root/shoot ratio and the proportion of fine roots in net production may decrease (Olsthoorn, 1991; Vogt et al., 1987).

This paper reports the results of attempts to analyse the relations of different methods (sequential coring and ingrowth cores) to study fine root biomass and NPP in Norway spruce stand of a high productivity. The sequential coring method has been the most common approach to determining fine root biomass and NPP in the field (Vogt and Persson, 1991), where both the biomass and necromass data reflect the natural status. For ingrowth cores, a stabilisation period is required, and older roots are missing in initially root-free soil volumes. Because the roots are still expanding into the ingrowth cores in the third year, comparison of absolute values between the two methods is difficult. Therefore, more relative parameters should be used: biomass:necromass ratio, root turnover rate, longevity, biomass and NPP proportions of different root diameter classes and the results received by different methods should be compared. On the basis of the data from the two methods, we tried to find a combined method for determining the NPP of fine roots that would be less time consuming.

We hypothesised, that the proportion of fine roots in the total biomass as well as in the annual NPP decreases as site conditions improve. We have studied fine root bio- and necromass, fine root annual NPP and also calculated turnover rate and proportion of fine root production in the NPP of trees of a Norway spruce stand of high productivity.

The objectives of our study were:

1) assessment of the NPP of fine roots in a high productivity spruce stand using different methods (sequential coring and ingrowth cores),
2) a comparison of methods regarding both the objectivity of root production estimation as well as labour intensity,
3) assessment of the share of fine roots in the NPP of the stand.

Material and Methods

Site and stand descriptions

The study was carried out in 60-year-old Norway spruce (Picea abies (L.) Karst.) stand in Roela (26° 45' E; 58° 46'N) in Estonia. The site type is *Oxalis* according to the classification of Paal (1997), site quality class I^a, and the soil type is Umbric Luvisol, according to the FAO classification (FAO-Unesco 1988). Stand characteristics are described by Lõhmus and Ivask (1995), where the stand is indicated as Voore 1.

Daily mean soil temperatures at a depth of 20 cm in Roela forest calculated by linear regression model on basis of soil temperatures at a depth of 20 cm in Roela forest and at the meteorological station, at Jõgeva (The Estonian Meteorological Institute). Precipitation was measured in the Saarejärve area of integrated monitoring (ICP Integrated Monitoring EE02), located 5 km from Roela study area. Mean monthly precipitation and soil temperature dynamics are presented for different years in Fig. 1. In Estonia, eight climatic seasons, are distinguished, where in addition, to the 4 main seasons — spring, summer,

autumn and winter — 2 intermediate seasons between autumn and winter (late autumn, early winter) and 2 between winter and spring (late winter, early spring) (Jaagus & Ahas, 2000). The beginning of early winter coincide with beginning of the fine root dormancy period: when daily maximum temperature remains below zero and first snow cover forms.The the beginning of early winter varied between 08–25 November during 1996–1999 and the start of thermal growing season (+5°C) varied between 7–28 April.

In the 40-year-old Roela stand, biomass allocation in the above- and below-ground part was estimated on the basis of 7 model trees (including excavation of 6 root systems) and 45 soil cores (Lõhmus and Oja, 1983). Tree root biomass data in the Roela stand are given in Table 1. The belowground part formed 17.2% of the tree biomass in the 40-year-old spruce stand (Lõhmus and Oja, 1983). The above and below-ground annual NPP were estimated as well, but not including fine roots (d<2 mm).

Root sampling and processing

Sequential soil coring and ingrowth core methods were used to estimate fine root (<2 mm) biomass and NPP. Fine root samples were taken during four consecutive growing seasons in 1996–1999.

Sequential cores. Twenty soil cores (volumetric samples, core diameter 38 mm) per sampling were taken monthly during the period June-1996 to June-1997 (totally 140). The soil cores were divided into seven layers by depth: forest floor, 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–30 cm, 30–40 cm.

Ingrowth cores. In December 1996, 192 mesh bags (∅ 40 mm, mesh size 6 mm) were installed in the study area in a regular pattern in two groups of parallel transects. 16 ingrowth cores were installed per transect; the distance between the ingrowth cores in the transect as well as between the transects was 1 m. Ingrowth cores were inserted into the soil to a depth of 30 cm from the surface of the forest floor. A 1-cm-thick forest floor layer was put on top of each core. Mesh bags were filled with root-free soil according to soil genetical horizons.

In the root ingrowth core method, a total of 105 ingrowth core samples (15 per sample) were collected during the growing seasons 1997–1999, once after one year and three times in the second and third years.

Sampling was carried out in November 1997, June, August and November 1998 and June, September and December 1999. As in the soil core method, ingrowth cores were divided into the depth layers, except for the 30–40 cm layer.

Sample processing. All obtained subsamples were transported to the laboratory and stored frozen (–18°C) until analysis. In the laboratory, spruce roots from samples taken by both methods were washed free of soil and separated into living and dead roots. Both living and dead roots were separated into two diameter classes: d<1 mm and 1 mm ≤ d <2 mm (further 1–2 mm). The dry

mass was determined after drying of fine root samples at 70°C to constant mass. Ash content was determined from composed fine root samples of month.

Data calculation

The total fine root production was calculated by balancing the living and dead root biomass compartments according to the decision matrix presented by Fairley and Alexander (1985):

		LIVE		
		increase	**decrease**	
			$\Delta B_{dead} > \Delta B_{live}$	$\Delta B_{live} > \Delta B_{dead}$
DEAD	**increase**	$P=\Delta B_{live}+\Delta B_{dead}$	$P=\Delta B_{live}+\Delta B_{dead}$	P=0
	decrease	$P=\Delta B_{live}$	P=0	

Root turnover rate (yr^{-1}) was calculated as annual root production (kg ha^{-1} yr^{-1}) divided by mean fine root biomass (kg ha^{-1}). Mean, minimum or maximum root biomass are used to calculate fine root turnover rate (Eissenstat and Yanai, 2002) We used the mean fine root biomass instead of minimum or maximum biomass to avoid large fluctuations during the vegetation period. Fine root longevity was calculated as the reciprocal of root turnover rate (yr^{-1}).

Fine root proportion in total NPP was calculated using fine root biomass data (Table 1.) determined twenty years ago at the same study area and the fine root turnover rate calculated in this study.

Statistical analysis

The normality of variables was checked by Lilliefors and Shapiro-Wilk's tests. The live/(live + dead) proportions for different root diameter classes were normalized by arcsin-transformation. Linear regression analysis was performed to estimate the relationship between normalised root variables and soil depth.

For estimating seasonal variability of fine root biomass and live/(live + dead) proportions multiple comparison of means was applied using Tukey test for unequal n.

In all cases, level of significance $\alpha = 0.05$ was accepted.

Results

Mean ash content

The mean ash content of fine root samples is a means to check contamination of root samples with soil particles, because it consists of the ash of root tissues and of the ash of adhered soil. The mean ash content for <1 mm and 1–2 mm living roots in both soil core and in ingrowth core samples was 16% and 6%, respectively. For dead <1 mm roots the mean ash content in soil core and in ingrowth core samples was 28% and 21%, respectively. Ash content in dead 1–

2 mm root samples averaged 6% for both methods. Ash content of living <1 mm root tissues was estimated as 5.7% (Lõhmus et al., 1995). Thus, not all soil particles were removed by washing, and we used 6% for the ash concentration in both living and dead fine root <1 mm and 1–2 mm diameter classes.

Biomass

The vertical distribution of Norway spruce fine roots. In the case of both methods, sequential cores and 3rd year of ingrowth cores, the majority (about 90%) of living fine roots as found to be located in the forest floor, or top 20 cm mineral soil horizon (Fig 2.). The impact of sampling time on relative vertical distribution of fine root biomass was insignificant ($p > 0.05$) in all cases.

In the 3rd year after establishment, the fine root biomass in ingrowth cores had not reached the level inherent to the stand (Fig 2.).

We used the share of living fine roots of the total fine root mass (live + dead) as an indicator of fine root vitality in different soil layers. The share of the fine root biomass in the total fine root mass decreased with increasing soil depth in both the d<1 mm and d = 1-2 mm root fractions. The relationship between share of fine root biomass (y) and soil depth (x, cm), expressed for different root diameter classes, method (soil and ingrowth core) and year, is presented in Table 2. As no difference was found between the slopes (b) in Table 2, except 1-2 mm roots in 3rd year ingrowth cores, it indicates the similar impact of soil conditions on fine roots of different diameter classes. Negative slopes in Table 2 indicate the deterioration of soil conditions with increasing depth, which is typical of many soils.

The ingrowth dynamics of fine roots. The mean biomass in ingrowth cores, collected in third year after installation, was two times smaller than in soil cores (Table 3). Total mass of fine roots <2 mm in ingrowth cores formed 440 kg ha^{-1} at the end of the first year, and the share of dead roots was only 1,5%. The mean share of dead roots of the <2 mm root mass was 19 % and 49% in the second and third year, respectively. The 3rd year value is close to that obtained by sequential coring, where dead roots formed 52% of the fine, d<2 mm, root mass.

In the third year the mean biomass:necromass ratio in the ingrowth cores stabilised to the same level as in the soil cores (Table 3), moreover, when comparing the same soil layers in both methods (i. e. separating out the 30-40 cm layer in soil cores), the ratios converge even more, being 0.99 in soil cores and 1.05 in ingrowth cores.

In soil cores the share of <1 mm living roots formed 2/3 of the <2 mm root biomass; that is similar to the ratio in 3rd year ingrowth cores (62 %) (Table 3.).

Seasonal variability of <1 and 1–2 mm root biomass. The mean share of living fine roots in the total (live + dead) fine root mass varied seasonally for both <1 mm and 1–2 mm diameter classes (Fig 4A and 4B).

Comparing the monthly values in Fig 4A and 4B to the annual mean, the biggest negative difference was observed in November; the positive difference was the greatest in August and June, for <1 mm and 1–2 mm roots, respectively. The mean live/(live+dead) ratios are closest to the annual mean in September – October (Fig 4A and 4B), which is something that should be borne in mind if fine root samples for fine root biomass assessment in a stand are gathered only once a year.

NPP

The fine root (<2 mm) production estimated by the two methods was different: by sequential core method it was 2.5 t ha^{-1} yr^{-1} and by ingrowth core method, 0.9 t ha^{-1} yr^{-1} (second year after installation) and 1.0 t ha^{-1} yr^{-1} (third year after installation). The annual NPP estimated by ingrowth cores in the third year after installation was 2.5 times smaller than that by soil cores. The annual NPP estimates of different fine root diameter classes (<2 mm and <1 mm; 1–2 mm was calculated as their difference) are presented in Table 4.

Root turnover

According to the sequential cores the mean biomass of fine roots (<2mm) in the 60-year-old spruce stand was 1.4 t ha^{-1} and annual net primary production was 2.5 t ha^{-1} yr^{-1}; the calculated root turnover rate was 1.8 yr^{-1}. The root turnover rate in ingrowth cores in the third year was 1.4 yr^{-1}.

The inverse of the root turnover rate is, in turn, a measure of average root longevity (life expectancy), which, according to sequential cores and 3rd year ingrowth cores, is smaller for the finest roots (Table 5).

The share of fine roots in total net primary production

In the investigated spruce stand, at the age of 40-years the biomass of fine roots (d<2mm) was 1.64 t ha^{-1}. Using the turnover rate of fine roots found in the 60-year-old stand to estimate the fine root production in the 40-year-old stand, the estimate is 2.8 t ha^{-1} yr^{-1} (Tabel 6). Hence, in the highly productive spruce stand the annual net primary production of trees at the age of 40 year is estimated as 21.4 t ha^{-1} yr^{-1}, the share of the below-ground part forming 31%. Fine roots accounted for around 13.1% of the net primary production (Table 6).

Combined method to measure fine root production

Root research is very labour-intensive and, in order to reduce the volume of work we tried a combined method for the estimation of fine root production. Since the relative measures in ingrowth cores (Tables 3, 4, 5.) stabilised by the third year, the fine root production was calculated by multiplying the turnover rate of ingrowth cores by the fine root biomass estimated by soil coring (Fig 5).

According to our analysis for different diameter classes, the best time to collect fine root samples for biomass data is the autumn. The average share of living root in the study plot was closest to the annual mean for samples

collected in September - October (Fig 4A and 4B). For the fine roots (d<2 mm), the biomass was on the same level from June to October, with the values in June smaller, but statistically insignificantly so (Fig 5). The fine root biomass is significantly lower in November.

The suggested combined method gave an 11% higher estimate for fine root biomass in October and an 8% smaller estimate for fine root biomass in September of fine root measured annual NPP in our study stand (see Fig 5). According to Fig 5., is suitable for assessing fine root biomass in the vegetative period in any month, nevertheless in the given climatic conditions, biomass is smaller in June. At that time the detrimental effects of winter can not be ruled out and the intensive allocation of assimilates to growing shoots is in progress.

Discussion

The commonly used definition of fine roots as those with <2 mm diameter is problematic for many tree species because it lumps together populations of roots that cycle carbon at significantly different rates (Gaudinski et al., 2001; Gill and Jackson, 2000; Wells and Eissenstat, 2001). It has been suggested in the published literature that the term "fine roots" is a mix of static and dynamic root fractions and that the size class below which root properties and function shift from those of perennial to ephemeral is much smaller than the commonly used 2.0 mm diameter class (King et al., 2002). Our results clearly showed the differences in vertical biomass distribution (Fig 2), growth pattern in root-free soil (Table 2), amount of biomass and annual NPP (Table 3 and 4) between roots separated by diameter into classes <1mm and 1–2 mm. According to Wells and Eissenstat (2001), we support the use of a functional fine root definition based on the considerable differences in morphology, function and life history.

Fine roots of 1–2 mm in diameter are mostly with a secondary structure, which is functionally significant for the spreading and stability of the fine root system and for nutrient transport within the plant. Woody roots of diameter 1–2 mm develop from long roots with a primary structure, which occupy free soil volume quickly and create the option to form more absorbing roots on them later. Long roots occupied the root-free soil volume in ingrowth cores first. 1–2 mm roots constituted on average 49% of all fine roots at the end of the second year and 38% at the end of the third year. The mean percentage of 1–2 mm roots in soil cores was 34% (Table 3). Also, the NPP of long roots in ingrowth cores was bigger in the second than in the third year (Table 4). According to Lõhmus et al. (1986 and 1991) the yearly average increment of long roots with primary structure in Roela study area was 295 ± 53 mm, and their growth was seasonally more rapid from mid-June until mid-July. In the first half of the growth period the growth of long roots depended most on soil temperature, and in the second half, on the soil water content (Lõhmus et al., 1986, 1991).

The vertical distribution of Norway spruce fine roots

The similar vertical distribution of fine roots (<2 mm) in soil cores and ingrowth cores demonstrates that root vertical distribution is primarily governed by the gradients of soil characteristics. Fine root location in the upper part of the soil profile seems to be influenced by the availability of nutrients in the soil (Sainju and Good, 1993; Schmid and Kazda, 2002).The highest fine root biomasses were found in the upper part of the soil profile, where the concentration of nitrogen and organic matter as well as the activity of soil microbial communities were higher (Truu et al., 2001). The vertical distribution of the finest roots tends to be more shallow than that of long roots; about 78% of <1 mm root biomass and about 61% of 1–2 mm root biomass were found to be located in the forest floor — 10 cm mineral soil horizon. All short roots in Roela study area are ectomycorrhizas (Ostonen and Lõhmus, 2003), functionally adapted to the uptake of water and mineral nutrients. They belong to the <1 mm diameter class as the mean diameter of short roots was 0.42 ± 0.07 mm (Ostonen et al., 1999).

Negative slopes in Table 2 indicate the deterioration of soil conditions with increasing depth, something that is typical of many soil types. Our results are in good accordance with those of Strober et al. (2000), who found that the share of live fine roots in the total fine-root mass falls from 61% in the upper 0–5 cm layer to 22% at a depth of 20–40 cm. The vertical distribution of the proportion of living fine roots in the soil characterises on one hand the vitality of the roots and on the other, the rate of decomposition of the dead roots in the soil profile. The decomposition dynamics of the finest roots was studied in Roela Norway spruce stand in 1986–1990 under the forest floor and at depths of 10, 20, 30 and 40 cm; no significant differences in the remaining mass were found between the samples from various depths (Lõhmus et al., 1991; Lõhmus and Ivask, 1995). Hence, in present study the vertical distribution of the percentage of live fine roots most probably indicates the different vitality of the finest roots at various depths.

Biomass, production and its proportion of NPP

Comparison of the results obtained by sequential soil coring and ingrowth core methods showed that in the third year after the insertion of the ingrowth cores the natural fine root structure disturbed by the insertion began to reestablish in the initially root free soil. The fine-root biomass in the ingrowth cores was 2x smaller than in the natural soil, due to the absence of older roots. The proportions of different diameter roots in ingrowth cores was similar to that in sequential soil cores. <1 mm diameter roots formed 2/3 of the biomass of all the <2 mm roots (Table 3.). The relation between different fine root diameter classes is in good concordance with Cronan (2003), who measured the biomass of fine roots (<1 mm) and small roots (1–3 mm) in a 55-year-old Norway spruce stand, and found the mean proportion of <1mm fine roots of the <3mm root biomass to be 64%. The proportion of different diameter classes was

investigated in Roela in a 40-year-old stand (Lõhmus and Oja, 1983; Lõhmus et al., 1991; Table 1); the biomass of fine roots <1 mm was 1.3 t ha^{-1} and formed 66% of the <3 mm root biomass. In a deciduous forest the share of biomass of <1 mm roots of the <2 mm biomass was ~92% (calculated from Fahey and Hughes, 1994), hence species- or genera related differences in proportion of different diameter roots are not excluded.

The annual NPP in ingrowth cores collected in the third year after installation was 2.5 x smaller than in soil cores, however, some essential relative measures were already stabilised, for instance the biomass:necromass ratio (Table 3), the share of finest roots (<1 mm) in fine root biomass and production (Table 3, 4), and mean longevity (Table 5). The annual NPP of <1mm roots makes up 73% of the annual NPP of <2 mm roots and this is in good accordance with Cronan's (2003) findings, in which the NPP of <1 mm roots made up 71% of the annual NPP of <3 mm roots.

Root turnover is an important sink for plant primary production. In soil cores the root turnover was 1.8 times the biomass of <2 mm roots, but, 1.4 times the biomass in ingrowth cores in the third year. The difference may most probably be explained by differences in the vegetation periods as well as by the fact that in ingrowth cores all roots (including the longer-living roots with a secondary structure) were younger than 3 years.

The fine root life span values (Table 5) are in good concordance with minirhizotron data, whereby the methodology used for calculating longevity of roots presents median root life span, not the average (Burton et al., 2000; King et al., 2002; Majdi et al., 2001). We compared our results to corresponding published data for coniferous trees and found that significant differences in root life spans existed among sites (Table 7).

The large variance in root longevity, including that within one tree species, may result from differences in environmental conditions, from the age of the stand, from the used field method, or the calculation method. Different authors stress different factors that effect individual root longevity: soil microsite conditions (Joslin et al., 2000; Pregitzer et al., 1993, 2000), root development patterns (Marshall and Waring, 1985), length of growing season, and plant mineral nutrient conservation (Eissenstat and Yanai, 1997). Nadelhoffer (2000) hypothesized that fine root turnover and production increase by increasing N availability. Conversely, Finer and Laine (1998) showed that the turnover rate of pine roots was not significantly affected by the nutrient level of the site type. Table 7 reveals a tendency for finer roots to have a shorter life span. Root type (coarse, fine or mycorrhizal) is significant in affecting turnover rate and therefore average root life span (King et al., 2002). Majdi et al. (2001) concluded that mycorrhizal root longevity also depends on the branching orders of mycorrhizal roots.

The calculated estimate of fine root (<2 mm) NPP in the 40-year-old Roela stand was 2.8 t ha^{-1} yr^{-1} and the respective share of fine roots in the net primary production of the stand was around 13% (Table 6). This value is relatively small

compared to the results revealed in most studies, and the reason is most probably related to favourable soil conditions.

In literature, fine roots constituted 8 to 76% of the annual total NPP (Fogel 1985; Grier et al., 1981; Gower et al., 1996; Helmisaari et al., 2002; Keyes and Grier, 1981; Nadelhoffer and Raich, 1992; Persson, 1993). Gower et al. (2001) tried to summarize NPP and carbon allocation patterns for boreal forests in relation to climatic and biological variables, based on knowledge obtained from SWECON and BOREAS; fine roots accounted for 19 to 50% of the total NPP (Gower et al., 2001). However, according to SWECON, the C flux into the belowground system via the tree stem was 63% of net photosynthetic production (Ågren et al., 1980). Högberg et al. (2002) stated that the C allocation from the NPP to the root system is overestimated and that this raises many questions: how do the assimilates become divided belowground within roots and microbial associations (including ectomycorrhizal fungi), how to separate the respiration of roots and microbial communities. We found that the activity and diversity of microbial communities in the Norway spruce stands and pot experiments was always significantly higher in the soil-root interface than in bulk (Truu et al., 2001 and personal communication), which indicates the support of rhizosphere microbes by trees.

Keyes and Grier (1981) compared the proportion of fine roots in the total NPP of Douglas firs for two sites: low and high productivity, the results were 36.4% and 7.9%, respectively. This is in accordance with the general concept that in unfavourable site conditions more NPP is allocated to roots (Olsthoorn, 1991; Palumets, 1991; Vogt et al., 1987).

Vogt et al. (1998) revealed that one methodological approach could consistently result in higher or lower values when we use different calculation approaches for the data. We suggest the use of more stable relative measures to compare the different methods or approaches. The combined method for estimation of fine root NPP gave good results and was less labour-intensive. The suitability of the combined method for wider application requires further analysis.

The conclusion of Tingey et al. (2002), that sampling interval influences estimates of the fine root production and mortality, applies not only to the minirhizotron method but can be generalised to all sequential sampling methods such as sequential coring and in-growth cores. They suggested that if the interval between sampling periods is a significant proportion of the root life span, then part of the new fine roots will be born and will die between sampling intervals and their biomass fraction will not be included in estimates of fine root production and mortality. The average sampling interval in our study was 28 days by the sequential core method and 70–109 days by the ingrowth core method; this makes 14% and 27–42% of the mean fine root (<2 mm) life span, respectively. Hence, part of the newly-emerged nonsenecent root tips may die and decompose during the interval. Most finest roots (<1mm) decomposed slowly in the study area; after five years the mass loss was 40 % (Lõhmus et al., 1995).

Fungal biomass in fine roots

The carbon cost of fungal or bacterial symbionts in root systems should be included as part of field estimates of belowground production, but is not, because of sampling difficulties (Vogt et al., 1998). Data on how much fungal tissues incorporated in ectomycorrhizas contribute to biomass for field grown tree roots, and information on the carbon cost of the fungal partner incorporated into ECM tissues are still scarce (Hobbie and Colpaert, 2003; Lindahl et al., 2002; Rousseau and Reid, 1989).

We have made an attempt to scale fungal biomass in fine roots to the ecosystem level. The mean mass of one ectomycorrhizal tip and the number of tips per ha were estimated. Accordingly, as the mean tip mass was 0.079 ± 0.007 mg and the mean number of living tips was $4.5 \times 10^9 \pm 0.5 \times 10^9$ per ha, the mean mass of ectomycorrhizas per ha is calculated by multipling the values; the result is 355 kg. The proportion of the fungal mantle of an ectomycorrhizal root was estimated at 18% in this study area (Ostonen and Lõhmus, 2003). Making the obviously simplifying assumptions that 1) the share of fungal mantle does not change between vegetation periods, 2) the tissue density within ectomycorrhizas is homogeneous, 3) carbon cost for a mass unit of plant and fungus in ectomycorrhizas is equal, we can estimate the share of fungal biomass in fine root biomass and NPP. The biomass of fungus is (18% x 355 kg)/100% = 64 kg. Ectomycorrhizas formed, on average, 39% of the finest (<1mm) roots. If the annual NPP of finest roots (<1 mm) is 1830 kg ha^{-1} yr^{-1}, then, proportionally, that makes about 714 kg ha^{-1} yr^{-1} for ectomycorrhizas, and accordingly 18%, or about 129 kg ha^{-1} yr^{-1}, is used up for the ectomycorrhizal mantle. These calculations are very rough, since, first of all, we have made some very audacious assumptions and, secondly, we have not taken into account the intraspecific variations in the ecyomycorrhiza-forming fungi. Since mycorrhizal groups vary in growth rate and tissue quality, these changes in species assemblages could produce unforeseeable impacts on the productivity, survivorship, or decomposition of mycorrhizal biomass (Treseder and Allen, 2000).

Concludingly, comparing two different methods (sequential cores and ingrowth cores) used to estimate the fine root bio- and necromass, NPP, turnover rates, and longevities, the mass and NPP values for 3rd year ingrowth cores were approximately two times smaller than those obtained by soil cores. In the studied area, soil cores and 3rd year ingrowth cores produced turnover rates and longevities of fine roots (<2mm) that were similar. For both methods the biomass and NPP of finest roots (<1 mm) formed approximately two thirds of that of <2 mm roots. The turnover rate for finest roots was approximately 2.0 times the biomass, and longevity was half a year. The estimates remain within the bounds of the published minirhizotron measurements.

Fine-root biomass was stable during the vegetation period. According to our results fine root biomass may be measured in any month in the vegetative

period, nevertheless, in the given climatic conditions, the biomass tended to be smaller in June, when the detrimental effects of winter cannot be ruled out and the intensive allocation of assimilates to growing shoots is in progress. The fine root biomass decreased after the end of the vegetation period, in Estonian climatic conditions, in November. From the methodological point of view it is essential to use relative measures from the absorbing-root scale through to the stand scale in order to better express the functional efficiency of the forest ecosystem. In addition, the relative root parameters enable different root research methods to be combined and less labour-intensive root study methods to be derived. The new combined method based on turnover rate of 3rd year ingrowth cores and fine root biomass estimated by soil cores gave acceptable results in the investigated stand and was less labour-intensive than sequential coring. The suitability of the combined method for wider application requires further analysis.

The more detailed classification of fine roots (diameter classes <1 mm and 1-2 mm in this work) enables a more thorough understanding of the fine-root production process. Our results clearly showed the differences in fine root vertical distribution, growth pattern in root-free soil, and the amount of biomass and annual NPP, between roots separated by diameter into classes <1mm and 1–2 mm. In further investigations, the differentiation of fine roots based on their function should be taken into consideration.

Acknowledgments

This study was supported by Estonian Science Foundation grant No 2487, No 3977 and No 4895. We thank Mr. Ilmar Part for revising the English text.

References

Aber JD, Melillo JM, Nadelhoffer KJ, McClaugherty CA, Pastor J. 1985. Fine root turnover in forest ecosystems in relation to quantify and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317–321.

Ahlström K, Persson H, Börjesson I. 1988. Fertilization in a mature Scots pine (*Pinus sylvestris* L.) stand — effects on fine roots. Plant Soil 106, 179–190.

Allen MF. 1991. The ecology of ectomycorrhizae. Cambridge, UK: Cambridge University Press.

Ågren GI, Axelsson B, Flower-Ellis JGK, Linder S, Persson H, Staaf H, Troeng E. 1980. Annual carbon budget for a young Scots pine. Ecol Bull 32, 307–313.

Burton AJ, Pregitzer KS, Hendrick RL. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125: 389–399.

Cronan CS. 2003. Belowground biomass, production, and carbon cycling in mature Norway spruce, Maine, U.S.A. Can. J. For. Res. 33, 339–350.

Eissenstat DM, Yanai RD. 1997. The ecology of root life span. Adv Ecol Res 27: 1–62.
Eissenstat DM, Yanai RD. 2002. Root Life Span, Efficiency, and Turnover. *In*: Y. Waisel, A. Eshel, U. Kafkafi (Eds.) Plant Roots:The Hidden Half. Third edition. Marcel Dekker, New York., 221–238.
Fahey TJ, Hughes JW. 1994. Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. J Ecol 82, 533–548.
Fairley RI, Alexander IJ. 1985. Methods of calculating fine root production in forests. In: Fitter AH (ed.). Ecological interactions in soil. Spec. Publ. Br. Ecol. Soc. 4, 37–42.
FAO — Unesco. 1988. Soil map of the world. Revised Legend. World Soil Resources Report 60, Rome, 119 p.
Finer L, Laine J. 1998. Root dynamics at drained peatland sites of different fertility in southern Finland. Plant Soil 201, 27–36.
Fogel R. 1985. Roots as primary producers in below-ground ecosystems. *In* Ecological Interactions in Soil. Special publication of the British Ecological Society, No 4. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. pp 23–36. Blackwell Scientific, Oxford, UK.
Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD. 2001. The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. Oecologia 129, 420–429.
Gill RA, Jackson R 2000 Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147, 13–31.
Gower ST, McMurtrie R, Murty D. 1996. Above-ground net primary production decline with stand age: potential causes. Trees 11, 378–382.
Gower ST, Krankina O, Olson RJ, Apps M, Linder S, Wang C. 2001. Net primary production and carbon allocation patterns of boreal forest ecosystems. Ecol Appl 11 (5), 1395–1411.
Grier CC, Vogt KA, Keyes MR, Edmonds RL. 1981. Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. Can. J. For. Res. 11, 155–167.
Helmisaari HS, Makkonen K, Kellomäki S, Valtonen E, Mälkonen E. 2002. Below- and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. Forest Ecology and Management 165, 317–326.
Haynes BE, Gower ST. 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. Tree Physiol 15, 317–325.
Hobbie EA, Colpaert JV. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytol. 157, 115–126.
Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
Högberg P, Nordgren A, Ågren GI. 2002. Carbon allocation between tree growth and root respiration in boreal pine forest. Oecologia 132, 579–581.
Jaagus J, Ahas R. 2000. Space-time variations of climate seasons and their correlation with the phonological development of nature in Estonia. Clim Res 15, 207–219.
Jones RH, Mitchell RJ, Stevens GN, Pecot SD. 2003. Controls of fine root dynamics across a gradient of gap sizes in a pine woodland. Oecologia 134, 132–143.
Joslin JD, Wolfe MH, Hanson PJ. 2000. Effects of altered water regimes on forest root systems. New Phytol 147, 117–129.

Keyes MR, Grier CC. 1981. Above- and belowground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can J For Res. Vol. 11, 599–605.

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherry P. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol. 154, 389–398.

Lindahl BO, Taylor AFS. Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests — towards a less 'phytocentric' perspective. Plant Soil 242, 123–135.

Lõhmus K, Oja T. 1983. K metodike izuchenja podzemnoi chasti drevostoev. (The methodology of studying below-ground part of trees.) (In Russian) Lesovedenie 4., 56–62.

Lõhmus K, Lasn R, Oja T. 1986. Rost kornej eli evropejskoj v zavisimosti ot pochvennykh uslovij. Pochvovedenie No 6. M., 89–97.

Lõhmus K, Lasn R, Oja T. 1991. The influence of climatic and soil physical conditions on growth and morphology of Norway spruce roots. In: Plant roots and their environment. McMichael, B. L. and Persson, H. (eds.), Elsevier Science Publishers B. V., 233–239.

Lõhmus K, Ivask M. 1995. Decomposition and nitrogen dynamics of fine roots of Norway spruce (*Picea abies* (L.) Karst.) at different sites. Plant Soil 168–169.

Lõhmus K, Ivask M, Ostonen I. 1995. Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils. *In*: The Finnish Forest Research Institute. Research Papers 537. *Eds*. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83–87.

Majdi H, Nylund JE. 1996. Does liquid fertilisation affect life span of mycorrhizal short roots and fine root dynamics? Plant Soil 185, 305–309.

Majdi H, Kangas P. 1997. Demography of fine root in response to nutrient applications in a Norway spruce stand in south-western Sweden. Ecoscience 4, 199–205.

Majdi H, Damm E, Nylund J-E. 2001. Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytol. 150, 195–202.

Makkonen K, Helmisaari HS. 1999. Assessing Scots pine fine-root biomass — comparison of soil core and root ingrowth core methods. Plant Soil 210, 43–50.

Marshall JD, Waring RH. 1985. Predicting fine root production and turnover by monitoring root starch and soil temperature. Can J For Res 15, 791–800.

Nadelhoffer KJ, Aber JD, Melillo JM. 1985. Fine-roots, net primary production and soil nitrogen availability: a new hypothesis. Ecology 66 (4), 1377–1390.

Nadelhoffer KJ, Raich JW. 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. Ecology 73, 1139–1147.

Nadelhoffer KJ. 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystem. New Phytol 147, 131–139.

Olsthoorn AFM 1991 Fine root density and root biomass of two Douglas-fir stands on sandy soils in The Netherlands: 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth. J. Agric. Sci. 39, 1, 61–77.

Olsthoorn AFM, Tiktak A. 1991. Fine root density and root biomass of two Douglas-fir stands on sandy soils in the Netherlands. 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth J Agric Sci 39, 61–77.

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

Ostonen I, Lõhmus K. 2003. Proportion of fungal mantle, cortex and stele of ectomycorrhizas in *Picea abies* (L.) Karst. in different soils and site conditions. Plant and Soil (in print).

Palumets J. 1991. Analysis of Phytomass Partioning in Norway Spruce. Scripta Botanica VIII. Trt. 95 p.

Persson H. 1978. Root dynamics in a young Scots pine stand in Central Sweden. Oikos 30: 508–519.

Persson HÅ. 1983. The distribution and productivity of fine roots in boreal forests. Plant Soil 71, 87–101.

Persson, H. 1993. Factors affecting fine root dynamic of trees. Suo 43, 163–172.

Pregitzer KS, Hendrick RL, Fogel R. 1993. The demography of fine roots in response to patches of water and nitrogen. New Phytol 125, 575–580.

Pregitzer KS, Zak DR, curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytol 129, 579–585.

Pregitzer KS, King JS, Burton AJ, Brown SE. 2000. Responses of tree fine roots to temperature. New Phytol 147, 105–115.

Read, D. 1997. Mycorrhizal fungi: The ties that bind. Nature 388, 517–518.

Rousseau JVD, Reid CPP. 1989. Measurement of Carbon Cost in Ectomycorrhizae. *In* Applications of Continuous and Steady-State Methods to Root Biology. Eds. J G Torrey and L J Winship. pp 183–196. Kluwer Academic Publishers, Dordrecht.

Sainju UM, Good RE. 1993. Vertical root distribution in relation to soil properties in New Jersey pinelands forests. Plant Soil 150, 87–97.

Schmid I, Kazda M. 2002. Root distribution of Norway spruce in monospecific and mixed stands on different soils. Forest Ecology and Management 159, 37–47.

Strober C, Eckart GA, Persson H. 2000. Root growth and response to nitrogen. In: Schulze E-D, ed. Carbon and Nitrogen Cycling in European Forest Ecosystems, Ecol Stud 142. Berlin; Springer, pp 99–121.

Tingey DT, Phillips DL, Johnson MG. 2003. Optimizing minirhizotron sample frequency for an evergreen and deciduous tree species. New Phytol. 157, 155–161.

Treseder KK, Allen MF. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. New Phytol 147, 189–200.

Truu J, Truu M, Lõhmus K, Ostonen I, Ivask M, Kanal A. 2001. Structure and activity of microbial communities in soil-root interface and bulk soil in coniferous and deciduous stands. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root, 402–403.

Vogt KA, Grier CC, Vogt DJ. 1986. Production, turnover and nutritional dynamics of above- and belowground detritus of world forests. Adv Ecol Res 15, 303–307.

Vogt KA, Vogt DJ, Moore EE, Fatuga MB, Redlin MR, Edmonds RL. 1987. Conifer and angiosperm fine-root biomass in relation to stand age site productivity in Douglas-fir forests. J. Ecol. UK, 75, 857–870.

Vogt KA, Persson H. 1991. Measuring growth and development of roots. *In:* Techniques and Approaches in forest tree ecophysiology. Lassoie JP, Hinckley ThM (Eds). CRC Press, Boca Raton FL, 1991, 599 pp. Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O´Hara J, Asbjornsen H. 1996. Review of root dynamics in

forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187, 159–219.

Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H. 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187, 159–219.

Vogt KA, Vogt DJ, Bloomfield J. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200, 71–89.

Wells CE, Eissenstat DM. 2001. Marked differences in survivorship among apple roots of different diameters. Ecology 82, 882–892.

Yin X, Perry JA, Dixon RK. 1989. Fine-root dynamics and biomass distribution in a Quercus ecosystem following harvesting. For Ecol Manage 27, 159–177.

Table 1. Biomass (t ha^{-1}) and proportion (%) of different root diameter classes in 40-year-old Norway spruce stand in Roela.

Root dia-meter class, mm	**<1**	**1–2**	**2–3**	**3–4**	**4–5**	**5–10**	**10–15**	**15–20**	**≥20**	**Stump**	**Total**
Biomass, t ha^{-1}	1.26	0.31	0.35	0.37	0.61	1.73	1.29	1.05	15.35	26.25	**48.26**
Proportion, %	2.61	0.65	0.72	0.77	1.26	3.59	2.69	2.18	31.8	54.4	**100**

Table 2. The arcsin-transformed proportions of living roots from total root mass (live+dead) (y), y = a + bdepth. R^2 — coefficient of determination, p — level of probability, n.s. — not significant, * — significant difference between slopes.

Dependent variable	**a**	**B**	**R^2**	**p**
Soil core, d<1 mm	0,52	–0,0089	0.40	0.000001
Soil core, d = 1–2 mm	0,98	–0,0112	0,10	0.01
Soil core, d<2 mm	0,60	–0,0087	0,32	0.00001
Ingrowth core, d<1 mm, 3rd year				n. s.
Ingrowth core, d = 1–2 mm, 3rd year	1,21	–0,0361*	0,72	0.0001
Ingrowth core, d<2 mm, 3rd year	0,70	–0,0147	0,28	0.02

Table 3. The mean bio- and necromass (± standard error) of Norway spruce fine roots (kg ha^{-1}), proportion of different diameter classes (%) and biomass:necromass ratio, estimated by soil cores and ingrowth cores (2nd and 3rd year).

Method	Biomass, kg ha^{-1}			Necromass, kg ha^{-1}			Biomass/ Necromass < 2 mm
	<1 mm	1-2 mm	total	<1 mm	1-2 mm	total	
Soil cores	**935 ± 120** 66%	**480 ± 60** 34%	**1420 ± 170** 100%	**1370 ± 95** 89%	**170 ± 30** 11%	**1540 ± 120** 100%	0.94
Ingrowth cores, 2. a.	**265 ± 120** 51%	**260 ± 120** 49%	**525 ± 240** 100%	**80 ± 40** 64%	**50 ± 20** 36%	**125 ± 50** 100%	6.16
Ingrowth cores, 3. a.	**430 ± 115** 62%	**270 ± 10** 38%	**700 ± 105** 100%	**570 ± 35** 83%	**120 ± 20** 17%	**685 ± 50** 100%	1.05

Table 4. The estimates of annual fine root NPP kg ha^{-1} yr^{-1} by different diameter classes estimated by soil core and ingrowth core methods.

Annual net primary production	<1 mm, kg ha^{-1} yr^{-1}	1-2 mm, kg ha^{-1} yr^{-1}	<2 mm, kg ha^{-1} yr^{-1}	<1 mm/ <2 mm, %
Soil cores	1830	680	2510	73
Ingrowth cores, 2. a.	450	440	890	51
Ingrowth cores, 3. a.	865	100	965	90

Table 5. The mean calculated longevity (yr) of fine roots.

Method	**Turnover rate (yr^{-1})**		**Longevity, yr**	
	<2 mm	<1 mm	<2 mm	<1 mm
Soil cores	1.8	1.9	0,57	0,51
Ingrowth cores, 2. a.	1.7	1.7	0,59	0,59
Ingrowth cores, 3. a.	1.4	2.0	0,73	0,50

Tabel 6. The annual NPP (t ha^{-1} yr^{-1}) and its proportions (%) in a middle-aged *Oxalis*-Norway spruce stand.

	Net primary production, t ha^{-1} yr^{-1}	Proportion, %
Aboveground part	14.7	68.7
Coarse roots, stump (d>2 mm)	3.9	18.2
Fine roots (d<2 mm)	2.8	13.1
Total	21.4	100

Table 7. Fine root longevities in different diameter fractions of coniferous trees. Investigation methods are indicated: SC — sequential coring, IC — ingrowth cores, MR — minirhizotrons.

Tree species	Stand age	Mycorrhizal roots, days	<1 mm, days	1-2 mm, days	<2 mm, days	Investigation method	Source	Comments
Picea abies	60	–	**192** **182**	**260** **912**	**203** **261**	SC IC, 3rd year	present study	High productive site.
Picea abies	55	–	**441**	–	–	SC	Cronan, 2003	NPP calculated by budget method and min-max difference approach
Picea abies	25	**750**^{I} **600**$^{0-20}$ **980**^{II} **764**$^{20-40}$ **400**^{III}**1000**$^{40-85}$	–	–	–	MR	Majdi et al., 2001	Median longevities; superscripts denotes different orders: I unbranched, II main axes of branched, III side branched of branched short roots; and soil horizons.
Picea abies	25	**240**	–	–	–	MR	Majdi & Nylund, 1996	Median longevity
Pinus sylvestris	15 35 100	–	–	–	**487** **166** **111**	SC	Helmisaari et al., 2002	
Pinus sylvestris	120	–	–	–	**281**	SC	Persson,1983	
Pinus sylvestris	30 >30 ~85	–	–	–	**273** **230** **201**	SC	Finer & Laine, 1998	Meso-oligotrophic tall sedge fen Oligotrophic tall sedge pine fen Ombrotrophic dwarf-shrub pine bog
Pseudotsuga menziesii	40	–	–	–	**703** **541**	SC SC	Keyes & Grier, 1981	High productivity site Low productivity site, NPP was estimated as annual biomass increment + annual losses
Pinus taeda	11	**388**	**181**	**285**	–	MR	King et al., 2002	

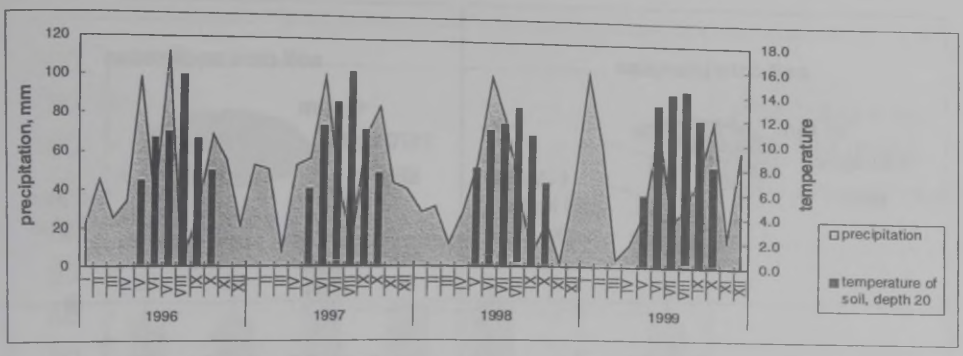

Fig 1. The variation of daily average temperatures (C°) at a 20 cm soil depth and the variation of monthly average precipitation (mm) at a distance of 5 km from the study site.

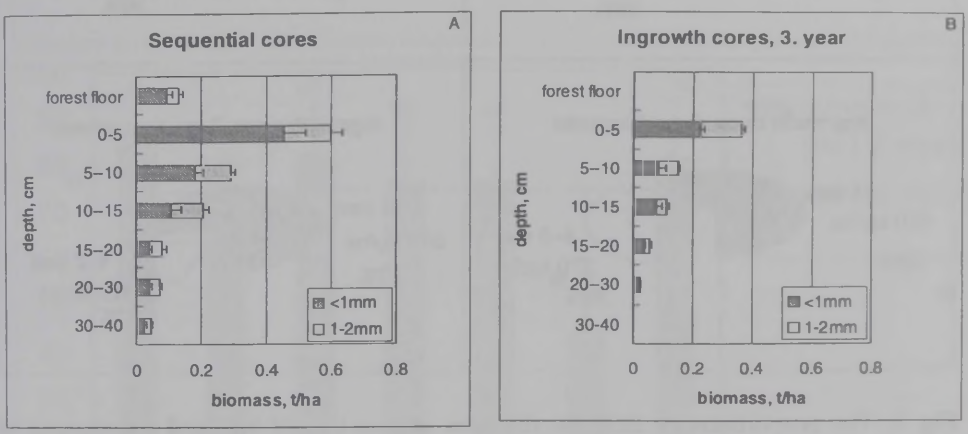

Fig 2. The vertical distribution of Norway spruce fine roots of different diameter classes in sequential cores and in ingrowth cores of up to 40 cm depth of mineral soil. Bars indicate standard errors.

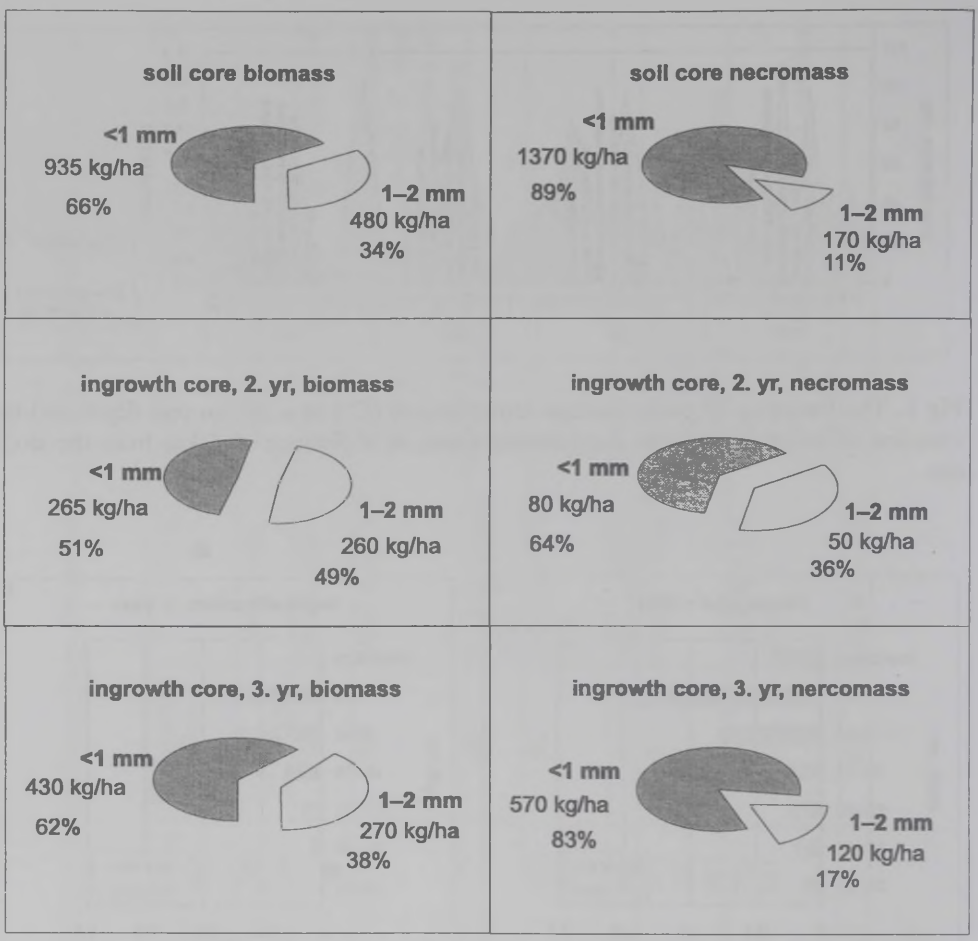

Fig 3. The proportions of different diameter classes (%) of bio- and necromasses of Norway spruce fine roots, estimated by soil cores and ingrowth cores (2nd and 3rd year).

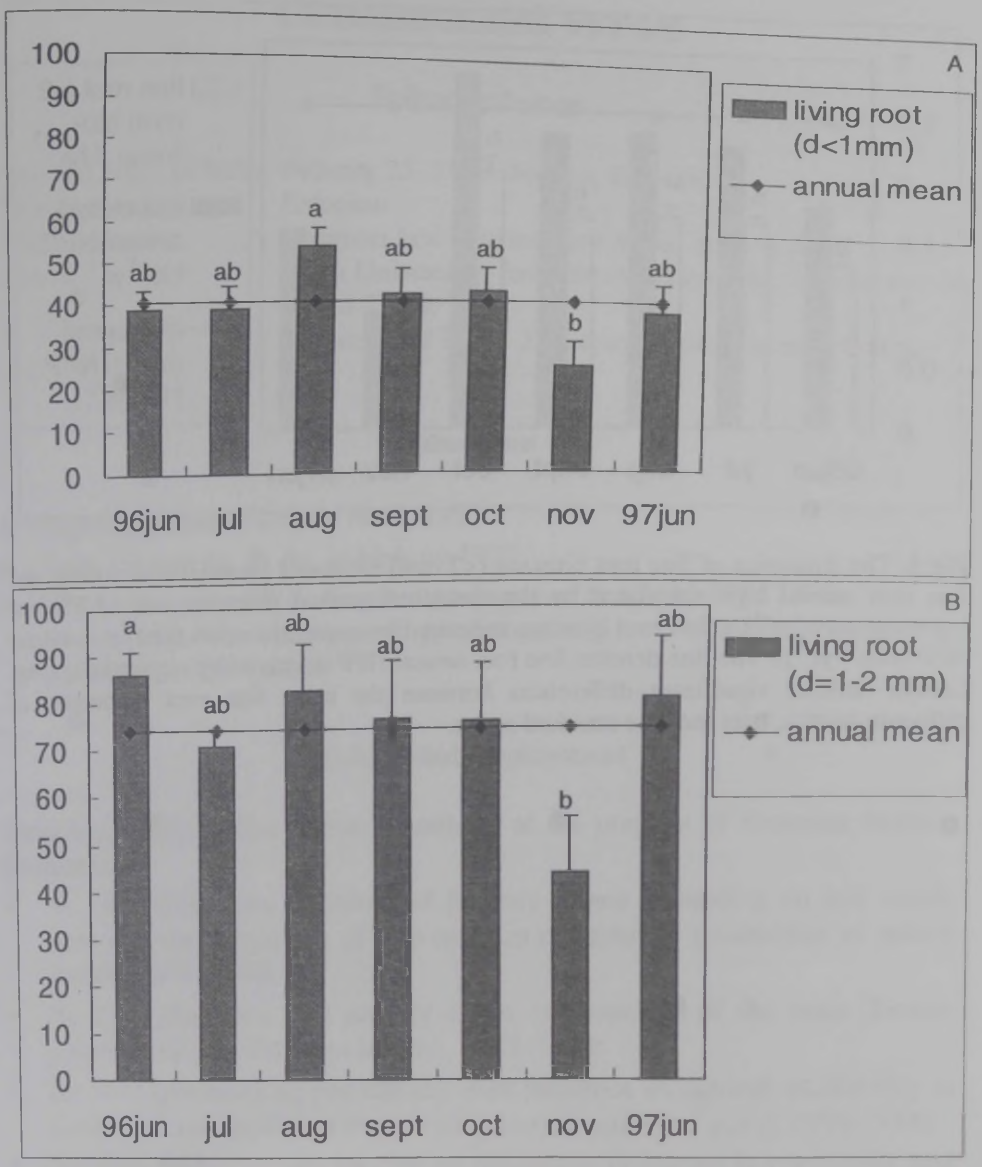

Fig 4. The seasonal variation in proportions of living fine root mass (d<1 mm, d=1–2 mm) within the total diameter class and the annual mean. Letters denote significant differences between the means of different months (Tukey test for unequal N, $p<0.05$). Bars indicate standard errors.

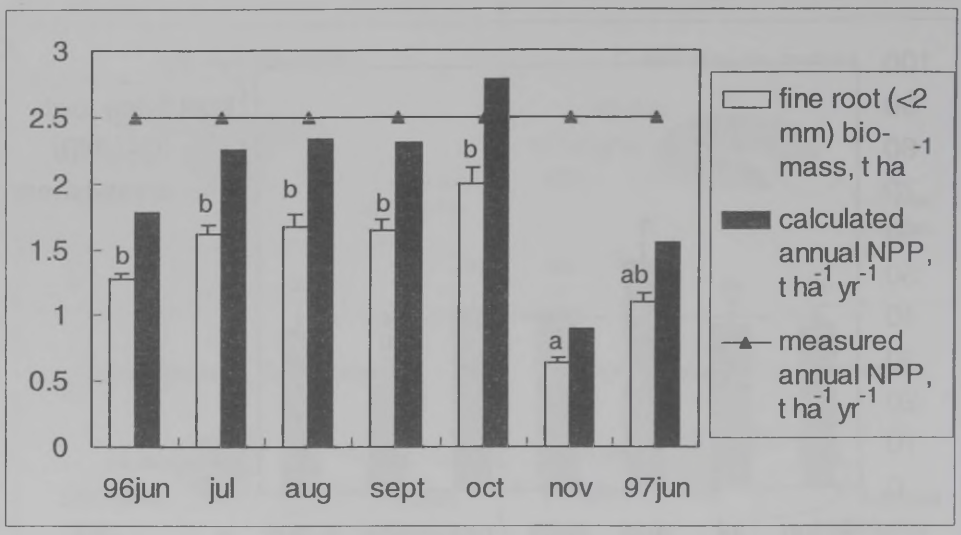

Fig 5. The dynamics of fine root biomass (<2 mm) estimated by sequential coring, and fine root annual NPP calculated by the combined method (turnover rate of 3rd year ingrowth core (yr^{-1}) x fine root biomass measured by sequential cores (t ha^{-1}) = annual NPP (t ha^{-1} yr^{-1})). The line denotes fine root annual NPP measured by sequential coring. Letters indicate significant differences between the mean fine root biomasses of different months, bars indicate standard errors.

CURRICULUM VITAE

Ivika Ostonen

Date and place of birth: Febrary 25, 1973, Jõgeva, Estonia.
Citizenship: Estonian
Marital status: common-law married, son Mikk, born in 2000
Address: Tartu University, Institute of Geography, Vanemuise 46, 51014 Tartu,
phone: +372 7 375 231, email: Ivika.Ostonen@ut.ee

Education

- Jõgeva Secondary School No 2, 1991
- Tartu University, B. Sc. in biology 1995
- Tartu University, M. Sc. in plant ecology and ecophysiology 1997. Dissertation: "The ecomorphology of absorbing roots of Norway spruce (Picea abies (L.) Karst.)."

Professional employment

Tartu University, senior research assistant at the projects of Estonian Science Foundation:

- No 2487 (Fine root dynamics of Norway spruce depending on soil conditions and the proportion of fine roots in net primary production of spruce stands), 1996–1998;
- No 2726 (Structure and activity of the communities of the main decomposers of litter in Estonian forests), 1997–1998;
- No 3977 (Rhizosphere process and their influence on nutrient availability in coniferous and deciduous forest ecosystems in different soils), 1999–2000;
- No 4895 (Effect of tree species on rhizospere processes in coniferous and deciduous seedlings in different soil conditions), 2001–2003.
- Estonian Forest Conservation Area Network, botanist, 1999–2001
- Tartu University, Institute of Geography, specialist of ecology on the SNS project nr 82 "Role of fine roots in carbon dynamics of forest soils", 2002 and joint project (Finnish Forest Research Institute, Vantaa Research Centre-University of Tartu) "The effect of ash+sludge application on tree mycorrhizas in forested mineral soil", 2002
- Tartu University, Institute of Geography, researcher, 2003 –

Additional studies

1994 Institute of Plant Sociology, Klagenfurt, Austria.
1995 Finnish Forest Research Institute, Helsinki, Finland.
1998 Individual Scolarship of Swedish Institute, the Visby Programme, Investigation of fine root anatomy collected from different forest ecosystems in Estonia in cooperation with Hans Persson's research group, Department of Ecology and Environmental Research, SLU, Uppsala.

Course: "Effects of environmental factors on nutrient cycles, below and aboveground production" SLU, Department of Ecology and Environmental Research, Uppsala.

Awards

First prize in realm of bio-geo science from national contest for student research 1997

Membership in societies

Estonian Naturalists' Society

LIST OF PUBLICATIONS

Articles in peer-reviewed journals

*__Ostonen I__, Lõhmus K and Lasn, R. 1999. The role of soil conditions in fine root ecomorphology in Norway spruce (Picea abies (L.) Karst.). Plant and Soil 208:283–292.

*__Ostonen I__ and Lõhmus K. 2003. Proportion of fungal mantle, cortex and stele of ectomycorrhizas in *Picea abies* (L.) Karst. in different soils and site conditions. Plant and Soil (in print).

*Ivask, M., Truu, J., Truu, M., Lõhmus, K., **Ostonen, I.** 1999. The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia. Baltic Forestry, 2: 32–36

*Ivask, M., Lõhmus, K., Truu, J., Truu, M., **Ostonen, I**. 2000. Earthworm Lumbricidae communities in alder and aspen forest: three case studies. Baltic Forestry 6, 1, 74–77.

*__Ostonen I__, Lõhmus K and Pajuste K. 200X. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce stand: comparison of soil core and ingrowth core methods. The Ecosystems. (submitted to Ecosystems)

Articles in proceedings

*Lõhmus K, Ivask M and **Ostonen I.** 1995. Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils. *In*: The Finnish Forest Research Institute. Research Papers 537. *Eds*. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83 – 87.

Ostonen, I., Lõhmus, K. 2001. The effect of soil conditions on the anatomy and morphology of Norway spruce (*Picea abies* (L.) Karst.) ectomycorrhizae. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root Research, 204–205.

Truu, J., Truu, M., Lõhmus, K., **Ostonen, I.**, Ivask, M., Kanal, A. 2001. Structure and activity of microbial communities in soil-root interface and bulk soil in coniferous and deciduous stands. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root, 402–403.

Publications in books

Viilma, K., Öövel, J., Tamm, U., Tomson, P., Amos, T., **Ostonen, I.**, Sørensen, P., Kuuba, R. 2001. Estonian Forest Conservation Area Network. Final Report of the Estonian Forest Conservation Area Network Project. Triip Grupp, Tartu. 95+306 p. (ed. J.Paal).

*Granhall, U., Lõhmus, K., Püttsepp, Ü., **Ostonen, I.** 200X. Mycorrhizae in *Alnus*. In: Eds. Ü. Mander and K. Lõhmus. Riparian alder forests: Their importance as buffer zones and bioenergy sources. Kluwer Academic Publishers, Dordrecht. (Accepted).

Publications in Estonian

Ostonen, I. 1995. Imijuurte eripind — ökofüsioloogiliste kasvumudelite parameeter. Juureökoloogia seminar., Lõhmus, K. (ed.). Trt., 45–48.

Ostonen, I. 1995. Teooriad, mis seletavad juurte gravitroopsust. Schola Biotheoretica XXI, "Äratundmise teooria", Kull, K., Möls, T., Trapido, T. (toim.), Teaduste Akadeemia kirjastus, Trt.–Tln., 58–63.

Ivask, M., Truu, J., Lõhmus, K., Truu, M., **Ostonen, I.** 1999. Mõnede lagundajakoosluste struktuur ja aktiivsus kuusikutes ja männikutes. Metsanduslikud uurimused XXXI:104–110.

Viilma, K., Öövel, J., Tamm, U., Tomson, P., Amos, T., **Ostonen, I.**, Sørensen, P., Kuuba, R. 2001. Eesti metsakaitsealade võrgustik. Projekti 'Eesti metsakaitsealade võrgustik' lõpparuanne. Triip Grupp, Tartu. 83+243 p. (Toim. J. Paal, I. Etverk).

* denotes publications used in the present thesis

CURRICULUM VITAE

Ivika Ostonen

Sünniaeg ja koht:	25. veebruar 1973, Jõgeva
Kodakondsus:	Eesti
Perekonnaseis:	vabaabielus, poeg Mikk, sünd. 2000
Aadress:	Tartu Ülikool, Geograafia instituut, Vanemuise 46, 51014 Tartu, telefon: 07 375 231, email: Ivika.Ostonen@ut.ee

Haridus

- Jõgeva II Keskool, 1991
- Tartu Ülikool, bioloogia eriala 1995, teaduste bakalaureus (BSc)
- Tartu Ülikool, taimeökoloogia ja ökofüsioloogia eriala 1997, teaduste magister (MSc). Magistritöö: "Hariliku kuuse imijuurte ökomorfoloogia"

Teenistuskäik

Tartu Ülikool, põhitäitja ETF grantiprojektides:

- nr 2487 "Hariliku kuuse peente juurte dünaamika sõltuvus mullatingimustest ja nende osatähtsus kuusikute netoproduktsioonis" 1996–1998;
- nr 2726 "Peamiste variselagundajate koosluste struktuur ja aktiivsus Eesti kuusikutes" 1997–1998;
- nr 3977 "Risosfääriprotsesside mõju mineraaltoitainete kättesaadavusele erinevatel muldadel kasvavates okas- ja lehtpuumetsades" 1999–2000;
- nr 4895 "Puuliigi mõju risosfääriprotsessidele erinevates mullatingimustes kasvavatel okaspuu- ja lehtpuuseemikutel" 2001–2003.
- Eesti Metsakaitsealade Võrgustik, botaanik, 1999–2001.
- Tartu Ülikool, Geograafi Instituut, ökoloogia spetsialist SNS projektis nr 82 "Role of fine roots in carbon dynamics of forest soils", 2002 ja Soome Metsauuringute Instituudi Vantaa urimiskeskuse ja TÜGI ühisprojektis "The effect of ash+sludge application on tree mycorrhizas in forested mineral soil", 2002
- Tartu Ülikool, Geograafia instituut, erakorraline teadur, 2003–

Erialane enesetäiendamine

1994 Taimesotsioloogia Instituut, Klagenfurt, Austria
1995 Soome Metsauurimise Instituut, Helsinki, Soome.
1998 Rootsi Instituudi Visby programmi stipendiaat Rootsi Põllumajandusülikoolis, Uppsala, Rootsi.
1998 Rahvusvaheline kursus: "Keskkonnafaktorite mõju metsade aineringele ja maapealse ja maa-aluse osa produktsioonile." Rootsi Põllumajandusülikool, Uppsala Rootsi.

Tunnustused

Esimene preemia Eesti üliõpilaste teadustööde 1997. a. riiklikul konkursil, geo- ja bioteaduste valdkonnas.

Teadusorganisatsiooniline tegevus

Eesti Loodusuurijate Selts

TEADUSLIKUD PUBLIKATSIOONID

Artiklid eelretsenseeritud ajakirjades

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

*Ostonen I and Lõhmus K. 2003. Proportion of fungal mantle, cortex and stele of ectomycorrhizas in *Picea abies* (L.) Karst. in different soils and site conditions. Plant and Soil (in print).

*Ivask, M., Truu, J., Truu, M., Lõhmus, K., **Ostonen, I.** 1999. The Earthworm Communities and Microbial Activities in Coniferous Forests of Estonia. Baltic Forestry, 2: 32–36.

*Ivask, M., Lõhmus, K., Truu, J., Truu, M., **Ostonen, I**. 2000. Earthworm Lumbricidae communities in alder and aspen forest: three case studies. Baltic Forestry 6, 1, 74–77.

*Ostonen I, Lõhmus K and Pajuste K. 200X. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce stand: comparison of soil core and ingrowth core methods. The Ecosystems. (submitted to Ecosystems)

Teesid ja artiklid konverentsiettekannetes

*Lõhmus K, Ivask M and **Ostonen I.** 1995. Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils. *In*: The Finnish Forest Research Institute. Research Papers 537. *Eds*. H-S. Helmisaari, A. Smolander and A. Suokas. Helsinki, 83–87.

Ostonen, I., Lõhmus, K. 2001. The effect of soil conditions on the anatomy and morphology of Norway spruce (*Picea abies* (L.) Karst.) ectomycorrhizae. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root Research, 204–205.

Truu, J., Truu, M., Lõhmus, K., **Ostonen, I.**, Ivask, M., Kanal, A. 2001. Structure and activity of microbial communities in soil-root interface and bulk soil in coniferous and deciduous stands. In: Proceedings of the 6th Symposium of the International Society of Root Research, November 11–15, 2001; Nagoya, Japan.Published by Japanese Society for Root, 402–403.

Artiklid raamatus

Viilma, K., Öövel, J., Tamm, U., Tomson, P., Amos, T., **Ostonen, I.**, Sørensen, P., Kuuba, R. 2001. Estonian Forest Conservation Area Network. Final Report of the Estonian Forest Conservation Area Network Project. Triip Grupp, Tartu. 95+306 p. (ed. J. Paal).

*Granhall, U., Lõhmus, K., Püttsepp, Ü., **Ostonen, I.** 200X. Mycorrhizae in *Alnus*. In: Eds. Ü. Mander and K. Lõhmus. Riparian alder forests: Their importance as buffer zones and bioenergy sources. Kluwer Academic Publishers, Dordrecht. (Accepted).

Eestikeelsed teaduslikud publikatsioonid

Ostonen, I. 1995b. Imijuurte eripind — ökofüsioloogiliste kasvumudelite parameeter. Juureökoloogia seminar., Lõhmus, K. (ed.). Trt., 45–48.

Ostonen, I. 1995c. Teooriad, mis seletavad juurte gravitroopsust. Schola Biotheoretica XXI, "Äratundmise teooria", Kull, K., Möls, T., Trapido, T. (toim.), Teaduste Akadeemia kirjastus, Trt.–Tln., 58–63.

Ivask, M., Truu, J., Lõhmus, K., Truu, M., **Ostonen, I.** 1999. Mõnede lagundajakoosluste struktuur ja aktiivsus kuusikutes ja männikutes. Metsanduslikud uurimused XXXI:104–110.

Viilma, K., Öövel, J., Tamm, U., Tomson, P., Amos, T., **Ostonen, I.**, Sørensen, P., Kuuba, R. 2001. Eesti metsakaitsealade võrgustik. Projekti 'Eesti metsakaitsealade võrgustik' lõpparuanne. Triip Grupp, Tartu. 83+243 p. (Toim. J. Paal, I. Etverk).

* tähistab artikleid, mida on kasutatud väitekirjas

APPROBATION

International conferences and meetings

1. "Dynamics of Physiological Processes in Woody Roots", Second International Symposium, 26–30 Sept. 1999, Nancy, France
Poster presentation: Ostonen I, Lõhmus K. Proportion of fungal mantle, cortex and stele of short roots in *Picea abies* (L.) Karst. in different soils.

2. "Canopy Dynamics and Forest Management — a Missing Link?" IUFRO Workshop, August 1–11, 1999, in Estonia, Finland and Sweden
Poster presentation: Ostonen I, Lõhmus K. Sensitivity of short root characteristics of Norway spruce (Picea abies (L.) Karst) to site productivity.

3. "Roots: the dynamic interface between plants and earth", The 6th Symposium of the International Society of Root Research, 11–15 November, 2001, Nagoya, Japan
Poster presentations:
1. Ostonen I, Lõhmus K. The effect of soil conditions on the anatomy and morphology of Norway spruce (*Picea abies* (L.) Karst.) ectomycorrhizae.
2. Truu J, Truu M, Lõhmus K, Ostonen I, Ivask M, Kanal A. Structure and activity of microbial communities in soil-root interface and bulk soil in coniferous and deciduous stands.

4. "Fine Root Turnover Workshop", Uppsala, Sweden, September 8–10, 2003.
Oral presentation: Ostonen I, Lõhmus K. Fine root turnover in a fertile middle-aged Norway spruce stand: comparison of soil core and ingrowth core methods.

Local conferences and meetings

1. Estonian VI Conference in Ecology "Sustainable development and natural life style", Tartu, 1994 (*Poster presentation*).
2. Workshop on Root Ecology, Tartu, 1995 (*Oral presentation*).
3. Estonian IX Conference in Ecology "Estonian Ecology in the Globalising World", Tartu, 2003 (*Poster presentation*)

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.

19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.
20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.

40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks.** Cholecystokinin (CCK) — induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and erotonin. Tartu, 1999, 123 p.
52. **Ebe Sild.** Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva.** Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna.** Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro.** Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane.** Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm.** Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg.** Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild.** The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.

60. **Niilo Kaldalu.** Studies of the TOL plasmid transcription factor XylS. Tartu 2000. 88 p.
61. **Dina Lepik.** Modulation of viral DNA replication by tumor suppressor protein p53. Tartu 2000. 106 p.
62. **Kai Vellak.** Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu 2000. 122 p.
63. **Jonne Kotta.** Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu 2000. 160 p.
64. **Georg Martin.** Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000. 139 p.
65. **Silvia Sepp.** Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira.** On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000. 96 p.
67. **Priit Zingel.** The role of planktonic ciliates in lake ecosystems. Tartu 2001. 111 p.
68. **Tiit Teder.** Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu 2001. 122 p.
69. **Hannes Kollist.** Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu 2001. 80 p.
70. **Reet Marits.** Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu 2001. 112 p.
71. **Vallo Tilgar.** Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002. 126 p.
72. **Rita Hõrak.** Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002. 108 p.
73. **Liina Eek-Piirsoo.** The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002. 74 p.
74. **Krõõt Aasamaa.** Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002. 110 p.
75. **Nele Ingerpuu.** Bryophyte diversity and vascular plants. Tartu, 2002. 112 p.
76. **Neeme Tõnisson.** Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002. 124 p.
77. **Margus Pensa.** Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003. 110 p.
78. **Asko Lõhmus.** Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003. 168 p.
79. **Viljar Jaks.** p53 — a switch in cellular circuit. Tartu, 2003. 160 p.

80. **Jaana Männik.** Characterization and genetic studies of four atp-binding cassette (ABC) transporters. Tartu, 2003. 140 p.
81. **Marek Sammul.** Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003. 159 p
82. **Ivar Ilves.** Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003. 89 p.
83. **Andres Männik.** Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003. 109 p.

ISSN 1024–6479
ISBN 9985–56–813–3