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ORIGINAL PAPER

# Fine-root rhizosphere and morphological adaptations to site conditions in interaction with tree mineral nutrition in young silver birch (*Betula pendula* Roth.) stands

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**Abstract** Limited nutrient acquisition from soil is a key process limiting productivity in boreal forest. We investigated short-root morphological adaptations and rhizosphere effect in relation to site conditions in interaction with tree mineral nutrition. We studied seven young (8- to 14-year-old) silver birch (*Betula pendula* Roth.) stands on abandoned agricultural land in Estonia. Soil pH varied from 3.8 to 7.0, and soil N % from 0.07 to 0.26%. Tree nutrient (NPK) status was expressed by leaf nutrient concentrations. Leaf N correlated negatively with short-root specific length and area. Summed activity (SA) and metabolic diversity of bacteria (by BIOLOG Ecoplate$^{TM}$), bacterial community diversity (by DGGE) and $pH_{KCl}$ were determined for rhizosphere (R) and bulk soil (S) to reveal the extent of the rhizosphere effect. Bacterial activity in rhizosphere was 1.4–4.7 times higher than in bulk soil. Ratio $SA_R/SA_S$ indicating root support to the rhizosphere bacterial communities decreased with increasing bulk soil pH; however, when bulk soil pH was $\geq$5, the decrease in $SA_R/SA_S$ was insignificant, i.e. the rhizosphere effect stayed at a stable level. Diversity of bacterial community was 6% higher in bulk soil than in rhizosphere. Rhizosphere acidification occurred in studied stands when bulk soil $pH_{KCl} \geq 5$. Short-root N % correlated positively with $SA_R/SA_S$. We concluded that tree N-nutritional status was related to short-root morphological parameters but not to studied microbiological characteristics in the soil of young silver birch stands.

**Keywords** *Betula pendula* · Fine-root morphology · Microbial activity · Microbial diversity · Mineral nutrition · Rhizosphere effect

**Abbreviations**

| | |
|---|---|
| BAS | Microbial basal respiration |
| CLPP | Community-level physiological profiles |
| DGGE | Denaturing gradient gel electrophoresis |
| MD | Metabolic diversity (Shannon index) by Biolog EcoPlates |
| R | Rhizosphere |
| PCA | Principal component analysis |
| S | Bulk soil |
| SA | Summed activity of cultivable bacteria, obtained with Biolog EcoPlates |
| SIR | Substrate-induced respiration |
| SRA | Specific root area |
| SRL | Specific root length |

## Introduction

The acquisition of limited nutrients from the soil is a key process limiting productivity and carbon sequestration in forest ecosystems. Nutrient uptake by roots is mediated by processes taking place in the rhizosphere, where, in comparison with the bulk soil, the biomass and activity of microorganisms are stimulated by root exudation and other fluxes of rhizodeposition (Berg and Smalla 2009; Jones et al. 2009; Lynch and Whipps 1990; Morgan et al. 2005).

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Also, microbial processes that support mineral nutrition, such as mineralization (Bader and Cheng 2007; Mueller and Shann 2007; Phillips and Fahey 2006; Zhao et al. 2007), and enzymatic activities, e.g. acid phosphatase activity (Liu et al. 2006; Radersma and Grierson 2004), are usually higher in the rhizosphere compared to the bulk soil. The phenomenon is well known as rhizosphere effect, which is considered to be the influence exerted by the growing plant on its immediate environment—in the rhizosphere (Lochhead and Rouatt 1955). Hence, rhizosphere effect can be measured as the ratio or the difference between rhizosphere and bulk soil parameters. If the ratio or difference is higher, the rhizosphere effect is higher and microbial processes in rhizosphere are more promoted and thus nutrient uptake by roots should be improved (Richardson et al. 2009).

Simultaneously with the rhizosphere effect, morphological adaptations of fine roots affect the capacity of roots to acquire nutrients from soil. The surface area of fine roots is important for the uptake of water and nutrients, and root length per unit of root biomass—specific root length (SRL)—is a key component of soil exploitation (Bauhus and Messier 1999) and efficient capture of P (Richardson et al. 2009). Both SRL and specific root area (SRA) are related to root efficiency because resource acquisition is proportional to the length or surface area, and root (construction and maintenance) cost is proportional to mass (Eissenstat and Yanai 1997; Lõhmus et al. 1989; Ostonen et al. 2007a). Morphological parameters of short roots are the best indicators of different root adaptation strategies because short roots are a functional compartment of the fine-root system; short roots have a living cortex, are commonly EcM colonized (in EcM species) and assimilate most nutrients (Guo et al. 2004; Ostonen et al. 2007b).

Soil resources are often poorly available to organisms due to the capacity of soil matrix to bind water and nutrients, so that roots have evolved to adapt and to influence their environment, optimizing their functional architecture to explore and make use of resources in heterogeneous soils (Hinsinger et al. 2009). Consequently, soil nutrient status and other soil properties are of central importance to root morphology as well as rhizosphere microbial population and carbon flow in the rhizosphere (Berg and Smalla 2009; Jones et al. 2009; Hodge et al. 2009).

In Northern Europe, birch (*Betula* sp) is commercially the most important broadleaved tree species (Hynynen et al. 2010). In Estonia, 31% of forests are birch [silver birch (*B. pendula* Roth.) and downy birch (*B. pubescens* Ehrh.)] stands. Silver birch is a fast-growing pioneer species, which, due to large seed fall and high seed dispersal, readily colonizes open ground. A dense-rooting, high fine-root biomass and foraging capacity (Curt and Prevosto 2003; Priha et al. 1999) as well as fast growth make silver birches competitive as a pioneer tree species in fertile sites including abandoned agricultural areas (Hynynen et al. 2010, Perala and Alm 1990). Due to changes in the political and economic situation, a clear increase in the amount of abandoned agricultural lands has been noted in Eastern Europe during the last two decades (Mander and Jongman 2000). About 300,000 ha of agricultural lands were abandoned in Estonia (Astover et al. 2006) during this time. Reforestation of abandoned lands has been proposed as a means to help offset increasing carbon emissions to the atmosphere via carbon accumulation in biomass and in soil (Richter et al. 1999) and to provide an alternative to fossil fuels for energy generation (Fearnside 1999). Hence, functioning of young silver birch stands on set-aside agricultural lands is an important issue for sustainable land use.

In alder (*Alnus glutinosa* (L.) Gaertn. and *Alnus incana* (L.) Moench) stands, the support provided to rhizosphere bacterial communities correlated positively with short-root SRA (Lõhmus et al. 2006a). However, in silver birch stands, the possible complex interactions between fine-root morphology, rhizosphere effect, soil attributes and tree mineral nutrition are still not fully understood.

The overall objective of this research was to quantify the response of short-root morphology and rhizosphere effect to different site conditions and to clarify whether and how it is related to tree nutrient status in young silver birch stands on abandoned agricultural land. Rhizosphere effect on bacterial activity and diversity as well as on pH was determined. Foliar NPK concentrations were used to reflect nutrient availability as well as mineral nutrition of trees. The specific aims of this research were (1) to quantify the variation in silver birch short-root morphological parameters at different sites and to assess sensitivity of root parameters to site conditions; (2) to find how leaf NPK and root N concentrations are related to short-root morphological parameters and rhizosphere effect; (3) to analyse the influence of soil characteristics on rhizosphere effect; and (4) to ascertain the possible interactions between short-root morphology and rhizosphere effect.

## Materials and methods

### Stand and soil characteristics

Fine-root morphological adaptations and the rhizosphere effect were studied in seven birch stands growing on abandoned lands with different soil conditions to ensure a wide spectrum of changes in structure and function of roots (Table 1). Stands are located throughout Estonia on various soils and were selected to represent a broad soil pH gradient (Table 2) because among soil parameters, soil pH has

**Table 1** Stand characteristics measured in 2002–2004 (Uri et al. 2007a, b, except Kassi and Reigi): location, soil type (IUSS Working Group WRB 2007), mean height (H), mean aboveground biomass of tree (BM) and annual precipitation (mean of 10 years 1997–2006)

| Stand | Location | Soil type | H (m) | BM/tree (kg) | Precipitation (mm year$^{-1}$) |
|---|---|---|---|---|---|
| Kassi | 58.23°N 25.63°E | Haplic planosol | 2.7 | n.e. | 753 |
| Väljaküla | 57.88°N 26.27°E | Stagnic luvisol | 4.4 | 1.58 | 730 |
| Lutsu | 58.03°N 27.15°E | Stagnic luvisol | 3.2 | 0.36 | 694 |
| Kambja | 58.23°N 26.73°E | Haplic albeluvisol | 6.6 | 2.69 | 657 |
| Haaslava | 58.28°N 26.97°E | Haplic cambisol | 3.7 | 0.63 | 637 |
| Pühatu | 58.93°N 24.53°E | Haplic cambisol | 4.2 | 1.97 | 708 |
| Reigi | 58.98°N 22.55°E | Rendzic leptosol | 1.4 | n.e. | 602 |

*n.e.* not estimated

**Table 2** Chemical characteristics of soil: $pH_{KCl}$, Kjeldahl nitrogen (N), available (lactate soluble) phosphorus (P) and loss on ignition (LOI); leaf NPK (Uri et al. 2007a, b, except Kassi and Reigi) and short-root N %

| Stand | Bulk soil | | | | | Leaf | | | Short root |
|---|---|---|---|---|---|---|---|---|---|
| | $pH_{KCl}$ | C/N | N (%) | P (mg kg$^{-1}$) | LOI (%) | N (g kg$^{-1}$) | P (g kg$^{-1}$) | K (g kg$^{-1}$) | N (g kg$^{-1}$) |
| Kassi | 3.8 | 14.0 | 0.12 | 40.4 | 2.8 | 27.7 | 3.5 | 10.0 | 24.3 |
| Väljaküla | 4.9 | 14.4 | 0.09 | 68.6 | 2.3 | 28.5 | 4.7 | 11.7 | 24.6 |
| Lutsu | 5.2 | 17.3 | 0.07 | 69.3 | 2.2 | 24.6 | 3.9 | 13.4 | 24.6 |
| Kambja | 5.2 | 14.8 | 0.09 | 60.8 | 2.4 | 27.8 | 3.9 | 10.8 | 22.4 |
| Haaslava | 5.7 | 15.6 | 0.12 | 62.4 | 3.1 | 25.1 | 3.8 | 9.3 | 23.2 |
| Pühatu | 6.9 | 12.6 | 0.16 | 72.2 | 3.4 | 25.9 | 3.7 | 10.7 | 22.6 |
| Reigi | 7.0 | 10.5 | 0.26 | 58.3 | 4.7 | 19.8 | 3.0 | 8.7 | 22.5 |

the largest effect on the diversity and richness of soil bacterial communities (Fierer and Jackson 2006). The age of the stands varied between 8 and 14 years. All fields were naturally regenerated, except Reigi, which was planted with bare-root two-year-old seedlings. Stand characteristics were measured in 2002–2004. By the time of soil and root measurements in 2006, tree density (no. of trees per ha) had converged during self-thinning. Precipitation data were taken from the nearest meteorological station.

## Sampling and processing of soil and roots

Ten initial samples from 0- to 20-cm soil layer ($20 \times 20$ cm$^2$) were collected randomly per stand in October 2006. A random fine-root ($d < 2$ mm) subsample was taken from each initial sample for morphological analysis. The remaining soil and roots from the samples of a stand were bulked to get a composite sample for microbiological and chemical analysis. A composite sample was formed and processed according to Gobran and Clegg (1996). Roots were carefully removed by hand from the field-moist mineral soil. Both the dead and coarse roots ($\geq$2 mm in diameter) were excluded. Living roots were differentiated from dead roots using visible criteria: resilience, texture, colour of bark and xylem (Vogt and Persson, 1991). The live fine roots were carefully shaken manually for 1 min in a plastic container to separate the soil aggregates from the roots. The fine roots with adhering soil gave the rhizosphere fraction. Mineral soil was passed through a 2-mm mesh sieve to give the bulk soil fraction.

## Short-root processing

Short roots were used to analyse root morphological adaptations of silver birch. Our study included only first- and second-order roots with living cortex. This fine-root compartment is functionally homogeneous and most active in water and nutrient uptake (Guo et al. 2004; Pregitzer et al. 2002).

Prior to measuring morphological parameters, the roots of 10 root samples per stand were washed with tap water to remove the soil particles. Two random short-root subsamples (15–22 living root tips) were taken per sample, 314–428 tips per stand. Short roots were considered alive if the exposed stele was still shiny and resilient (Vogt and Persson 1991). All studied root tips were, according to macroscopic features, ectomycorrhizal.

Short-root length, projection area and mean diameter of the sample were measured using WinRHIZO$^{TM}$ Pro 2003b (Regent Instruments Inc.). After measuring, short-root samples were dried at 70°C for 2 h (enough for short roots to get a constant mass) and weighed to an accuracy of

0.01 mg. The method for determining short-root morphological parameters (mean short-root length (mm), specific root area (SRA; $m^2\ kg^{-1}$), specific root length (SRL; $m\ g^{-1}$), tissue density ($kg\ m^{-3}$) and root tip frequency per 1 mg dry mass ($mg^{-1}$) is given in detail in Ostonen et al. 1999, 2007b.

## Chemical analysis

Leaf NPK was measured in 2002–2004. Leaf nitrogen concentration was measured by block digestion and steam distillation methods (Tecator AN 300). To measure leaf Kjeldahl phosphorus concentration, digest by flow injection analysis (Tecator AN 5242) and the analyser Fiastar 5000 (ISO/FDIS 15681) was used. Leaf potassium content was determined by flame photometry (Method 956.01), using Sherwood Model 425 Flame Photometer. Analyses were performed at the Laboratory of Biochemistry of the Estonian University of Life Sciences. The total N concentrations in short roots were determined using CHN analyser Perkin-Elmer 6400 Series II in the laboratory of the Department of Geology of the University of Tartu.

## Microbiological methods

We measured the rhizosphere effect on bacterial activity and community diversity as rhizosphere/soil (R/S) ratios and on pH as the pH difference between rhizosphere and bulk soil. Bulk soil and rhizosphere bacterial activity were determined by community-level physiological profile (CLPP) technique using the Biolog EcoPlate (Biolog Inc.) method. The CLPP method assesses the fast-growing cultivable part of the microbial community, which partly reflects the changes in total microbial community in rhizosphere (Söderberg et al. 2004). This technique has been proved to have discriminative power especially in the case of contrasting conditions and wide soil pH gradient (Lõhmus et al. 2006a). Biolog Ecoplates contain substrates that are known to be plant root exudates. One gram of fresh material from composite samples, composed of 10 subsamples taken randomly in a stand (to cover the within-area variability), was used in all cases. A 150-µl aliquot of a $10^{-4}$ dilution of the bulk soil was added to each of the 96 wells (31 carbon sources and control in three replicates) in the micro-plate. Plates were incubated at 22°C, and colour development was measured after 48 h for absorbance at 590 nm with optical density plate reader Multiscan 340. The data from Biolog EcoPlates were expressed as summed activity (SA) in optical density units per gram of dry mass. Higher ratio SA in rhizosphere divided by SA in bulk soil ($SA_R/SA_S$) is considered to indicate higher rhizosphere effect and greater support to the rhizosphere microbial communities (Lõhmus et al. 2006a). Metabolic diversity of the bulk soil ($MD_S$) and rhizosphere ($MD_R$) was measured as Shannon diversity indices, which were calculated according to following formula:

$$H' = -\sum p_i \ln p_i,$$

where $p_i$ is the proportional colour development of the $i$th well over total colour development of all wells of a plate.

The metabolic diversity ratio $MD_R/MD_S$ was computed.

Bacterial community diversity was measured using DNA-based denaturing gradient gel electrophoresis (DGGE) technique. Microbial DNA was extracted from soil samples with an UltraClean Soil DNA kit (Mo Bio Laboratories, Inc.). Bacterial community structure was assessed with 16S rDNA sequence-specific primer pairs using a semi-nested polymerase chain reaction (PCR). For the first amplification step, oligonucleotides p338f (Lane 1991) and p518r (Muyzer et al. 1993) were used as primers. For the second step, the product of the first PCR as a template and primers p338f GC and p518r were used. The GC clamp (40 bp) was added to the 338f primer to enable denaturing gradient gel electrophoresis. For the first PCR amplification, the isolated DNA (0.1 µl) was added as a template to a 20-µl reaction mixture. The PCR mixture included 1× PCR buffer (with $(NH_4)_2SO_4$), 200 µM concentrations of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 20 pmol of each primer and 0.5 U of Taq DNA polymerase (Fermentas). The second PCR amplification was performed in a total volume of 50 µl using 0.1 µl of the product from the first PCR as a template. Both PCR were performed in the same manner. After 5 min of denaturation at 95°C and 30 thermal cycles of 1 min at 95°C, 1 min at 53°C, and 45 s at 72°C, the PCR was finished by an extension step at 72°C for 10 min. A denaturing gradient gel electrophoresis system INGENYphorU-2 (Ingeny International) was used to separate the amplified gene fragments as recommended by the manufacturer. Approximately 35 µl of PCR products was applied for the DGGE analysis, and electrophoresis was performed as described by Muyzer et al. (1993) with 10% (vol/vol) polyacrylamide gel (acrylamide/bisacrylamide = 37.5:1 in 1× TAE buffer). A linear denaturing gradient of 40–65% was used. The DNA denaturing gradient was formed with deionized formamide and urea (100% denaturant agent is 7 M urea and 40% (vol/vol) deionized formamide). Gel was electrophoresed in 1× TAE buffer for 13 h at a constant temperature of 60°C and a constant voltage of 100 V. The gel was stained in 1× SyberGreen (Invitrogen) stain solution for 50 min in the dark. DGGE gel was digitized, and the banding pattern was analysed using the GelCompar II (Ver.4.0) program. The similarity in bacterial community between rhizosphere and bulk soil was expressed as Pearson $r$—the coefficient of correlation multiplied by 100%.

Bacterial community diversity, indicated by Shannon index, was calculated for rhizosphere and bulk soil.

Substrate-induced respiration (SIR) by Isermeyer technique was employed to measure metabolically active microbial biomass carbon. Glucose (0.4g/100 g soil) was added to 20 g of field-moist soil, and the mixture was incubated in a closed vessel for 4 h at 22°C in the dark. The produced $CO_2$ was absorbed in 0.1 M sodium hydroxide and quantified by titration. The microbial biomass C was calculated according to Beck et al. (1996). Soil microbial respiration rate (basal respiration—BAS) was measured by titration according to Öhlinger (1996); 20 g of soil was incubated in a closed vessel for 24 h at 25°C. The produced $CO_2$ was absorbed in 0.05 M sodium hydroxide, quantified by titration, and the respiration rate was calculated. The metabolic quotient (BAS/SIR), indicating carbon availability in the soil (Dilly et al. 2000; Weixin et al. 1996), was also calculated.

### Statistical methods

Lilliefors and Shapiro–Wilk's tests were applied to test the variables for a normal distribution. Except D and L, the variables were not normally distributed; for normalization, log and root transformations were applied. The homogeneity of the group variances was controlled by Levene's test; the group variances were inhomogeneous for root tissue density. Differences between stand means of short-root characteristics were checked by Tukey's test for unequal n for diameter, length, mass, SRL and number of tips per mass unit, and by Kruskal–Wallis test for tissue density. To compare leaf and short-root mean N concentrations as well as rhizosphere and bulk soil bacterial community diversities, pairwise *t* test was used. Sensitivity of a short-root parameter to varying site conditions was evaluated by the coefficient of variation of stand means. A higher coefficient of variation of a parameter was considered to indicate higher sensitivity to the site conditions. Principal component analysis (PCA) was applied for analysing short-root morphological parameters; soil pH, leaf NPK concentrations and precipitation data were used as supplementary variables. A significance level of $\alpha = 0.05$ was set in all cases. Within-site variation of soil chemical properties and leaf NPK concentrations was estimated as the relative standard error (the standard error divided by the mean and expressed as a percentage). Data management and analysis were performed using STATISTICA 7.1 (StatSoft Inc 2002) and CANOCO ver. 4.52 (ter Braak and Šmilauer 2002) software.

## Results

### Effect of site conditions on short-root morphology

Spatial variability of soil attributes within a stand was low. Relative standard error of soil pH, N and organic content remained below 10% in all stands. Relative standard error of leaf N, P and K was in all cases less than 5%. Bulk soil pH of studied stands varied from 3.8 to 7.0 and N % from 0.07 to 0.26% (Table 2). The highest pH, N % and loss on ignition (LOI) were measured in the soil of the Reigi birch stand. However, the leaf NPK concentrations were the lowest in Reigi.

Short-root morphology was significantly affected by site (Table 3); according to multidimensional gradient analysis (CANOCO RDA), forest site explained 30% ($P < 0.01$) of the total variability of short-root morphology. Mean coefficients of variation of stand means ranged as follows: number of tips per mass unit (35) > mass (32) > SRL (29) > SRA (22) > length (22) > tissue density (18) > diameter (12).

PCA analysis was applied to a number of short-root parameters, whereas site conditions were used as supplementary variables (Fig. 1a, b). The first two axes accounted

**Table 3** Mean short-root morphological parameters (±SE) in young silver birch stands on abandoned agricultural land in the year 2006: diameter, length, mass, specific root length (SRL) and shoot root tip frequency per unit short-root dry mass

| Stand | Diameter (mm) | Length (mm) | Mass ($10^{-6}$ g) | Tissue density (kg $m^{-3}$) | SRL (m $g^{-1}$) | No. of tips per mass unit ($mg^{-1}$) |
|---|---|---|---|---|---|---|
| Kassi | $0.328 \pm 0.006^{ab}$ | $0.94 \pm 0.06^{ab}$ | $13.1 \pm 0.9^{ab}$ | $166 \pm 7^{ab}$ | $74.8 \pm 4.3^{b}$ | $84.9 \pm 7.3^{b}$ |
| Väljaküla | $0.340 \pm 0.010^{ab}$ | $1.01 \pm 0.04^{ab}$ | $14.6 \pm 0.7^{b}$ | $162 \pm 5^{ab}$ | $70.6 \pm 3.3^{ab}$ | $71.0 \pm 3.2^{ab}$ |
| Lutsu | $0.355 \pm 0.011^{bc}$ | $0.96 \pm 0.05^{ab}$ | $13.6 \pm 0.8^{b}$ | $148 \pm 5^{a}$ | $72.1 \pm 4.4^{ab}$ | $78.0 \pm 4.9^{b}$ |
| Kambja | $0.368 \pm 0.011^{c}$ | $0.84 \pm 0.04^{a}$ | $13.5 \pm 0.7^{b}$ | $155 \pm 4^{a}$ | $63.3 \pm 3.1^{ab}$ | $77.7 \pm 4.1^{b}$ |
| Haaslava | $0.344 \pm 0.009^{bc}$ | $0.98 \pm 0.04^{ab}$ | $13.4 \pm 0.8^{b}$ | $148 \pm 5^{a}$ | $75.6 \pm 3.9^{b}$ | $78.4 \pm 4.2^{b}$ |
| Pühatu | $0.344 \pm 0.006^{bc}$ | $1.12 \pm 0.04^{b}$ | $19.5 \pm 1.4^{c}$ | $185 \pm 6^{b}$ | $59.9 \pm 2.9^{a}$ | $54.9 \pm 3.3^{a}$ |
| Reigi | $0.305 \pm 0.007^{a}$ | $1.01 \pm 0.05^{ab}$ | $9.9 \pm 0.7^{a}$ | $137 \pm 8^{a}$ | $106.6 \pm 5.6^{c}$ | $110.6 \pm 9.0^{c}$ |

Different letters indicate significant differences between means (by Tukey's test, $P < 0.05$), except root tissue density where Kruskal–Wallis test was used ($P < 0.0001$)

for 85.0% of the variation of short-root morphology (Fig. 1a, b). Mean SRA values varied between 64 and 102 $m^2\ kg^{-1}$ and were in a strong correlation with mean short-root specific length (SRA = 22 + 0.76SRL, $r^2 = 0.97$, $P < 0.0001$; Fig. 1b). Two significant correlations were found between site conditions and short-root morphology: leaf N % correlated with short-root SRL and SRA; $r = -0.83$; $P < 0.05$ in both cases. Leaf NPK vectors were oriented in the same direction in the PCA ordination plot; N and P correlated positively (one-sided test, $r = 0.75$, $P = 0.026$). Leaf NPK and mean annual precipitation tended to decrease with increasing short-root SRA and SRL (Fig. 1b).

Only one statistically significant correlation between soil chemical properties and short-root morphological parameters was revealed—short-root D correlated negatively with soil N % ($r = -0.81$, $P < 0.05$). Short-root N % was not related to short-root morphological parameters. Excluding Reigi, N concentration in short roots was lower than in leaves (pairwise $t$ test, $P < 0.02$). The variability of short-root N % in different stands was lower than the variability of leaf N % (coefficients of variation were 4.3 and 12.1, respectively).

## pH and microbiological characteristics of bulk soil and rhizosphere

The pH difference between bulk soil and rhizosphere of our stands varied between −0.2 and 1.5 and was higher in case of higher bulk soil pH; rhizosphere pH increased with increasing bulk soil pH ($r^2 = 0.85$, $P < 0.01$; Fig. 2). According to the linear regression line in Fig. 2, $pH_R$ was equal to $pH_S$ at pH = 4.3 and the rhizosphere was acidified when bulk soil pH was higher than 4.3. However, acidification occurred in studied stands when pH ≥ 5 (Fig. 2).

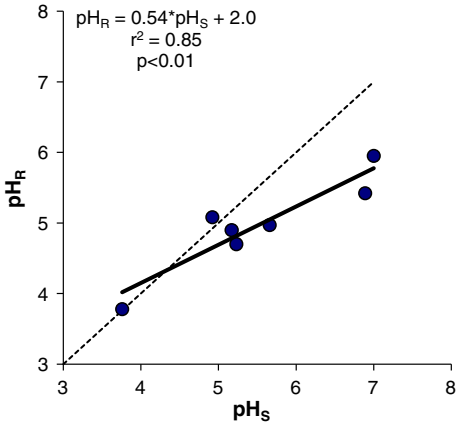


**Fig. 2** Relationship between rhizosphere pH ($pH_R$) and bulk soil pH ($pH_S$). *Dotted line* indicates line $pH_R = pH_S$. The rhizosphere was acidified (up to 1.5 units) when bulk soil pH was higher than 4.3

Both principal component analyses of microbial communities based on CLPP data (Fig. 3a) and on DGGE profiles (Fig. 3b) separated clearly rhizosphere and bulk soil samples. Compared to the rest of the studied stands, Kassi and Reigi had a higher similarity in bacterial community between rhizosphere and bulk soil according to DGGE profiles (Table 4). Bacterial community diversity according to DGGE data (Table 4) was 6% higher in bulk soil than in rhizosphere (pairwise $t$ test, $P < 0.01$).

Summed activity and metabolic diversity values of cultivable bacterial community were always higher for rhizosphere than for bulk soil (Table 4). Variation of summed activity between stands was three times higher in bulk soil than in rhizosphere (Fig. 3a; coefficients of variation were 44 and 15, respectively). Therefore, the summed activity ratio $SA_R/SA_S$ was affected more by bulk soil summed activity. Summed activity ratio $SA_R/SA_S$, which

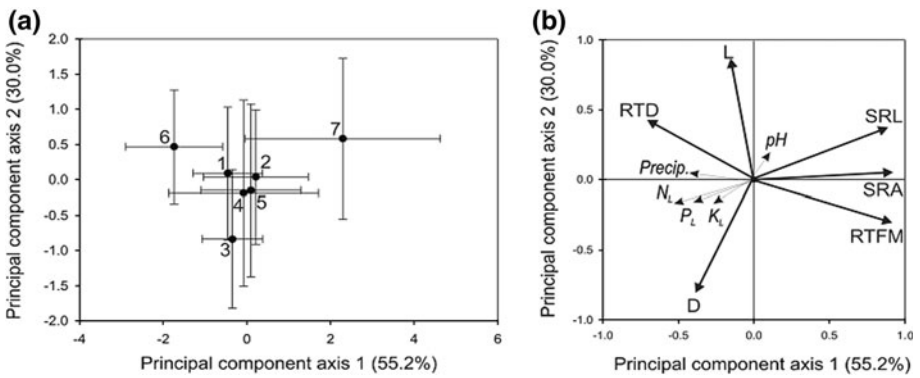


**Fig. 1** Principal component analysis based on correlation matrix of short-root morphological parameters. (**a**) Ordination of different stands by short-root morphological parameters along the first two PCA axes. Shown are mean and standard deviation values of sample scores for stands. Stands: *1* Väljaküla, *2* Haaslava, *3* Kambja, *4* Kassi, *5* Lutsu, *6* Pühatu, *7* Reigi. (**b**) Relationship of short-root morphological parameters with two first principal component axes. Abbreviations: *SRA* ($m^2\ kg^{-1}$) specific root area, *SRL* ($m\ g^{-1}$) specific root length, *L* (mm) short-root length, $RTF_M$ ($10^{-6}$ g) shoot root tip frequency per unit short-root dry mass ($mg^{-1}$), *RTD* ($kg\ m^{-3}$) root tissue density and *D* (mm) short-root diameter. Supplementary variables (indicated by dotted *line* and *italic*): soil pH, Precip.—annual precipitation (mean of 10 years 1997–2006; mm $year^{-1}$), $N_L$, $P_L$ and $K_L$—leaf *N*, *P* and *K* %, respectively

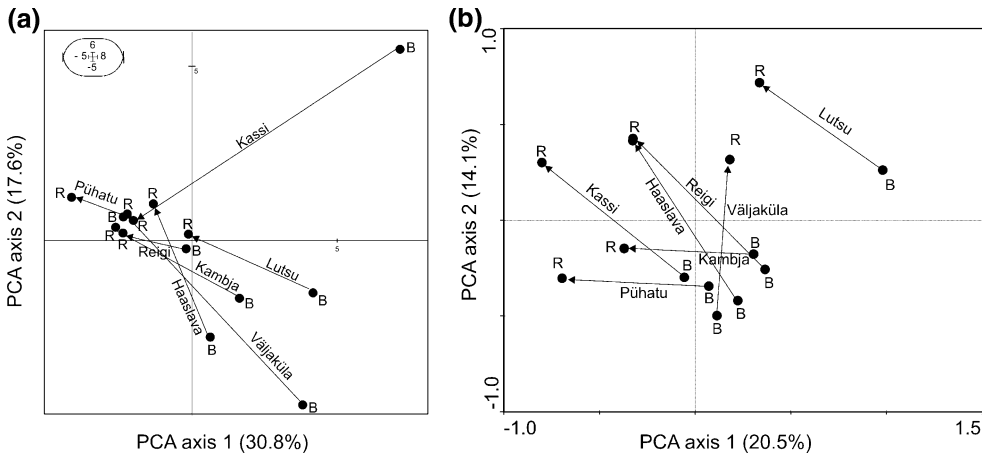


**Fig. 3** Ordination of rhizosphere and bulk soil samples based on principal component analysis of CLPP data (**a**) and the DGGE profiles of microbial communities (**b**). Letters *B* and *R* designate bulk and rhizosphere samples, respectively. An arrow connects bulk and rhizosphere samples from same location

**Table 4** Microbiological characteristics in bulk soil (S) and rhizosphere (R) of studied birch stands: microbial biomass (SIR), basal respiration (BAS), summed activity ratio $SA_R/SA_S$, metabolic diversity ratio $MD_R/MD_S$, similarity between R and S bacterial communities, and bacterial diversity (BD) ratio $BD_R/BD_S$

| Stand | Bulk soil microbial respiration | | Biolog data | | DGGE data | |
|---|---|---|---|---|---|---|
| | SIR (mg $C_{mic}$ $g^{-1}$) | BAS (mg $CO_2$ $g^{-1}24$ $h^{-1}$) | $SA_R/SA_S$ | $MD_R/MD_S$ | Similarity between R and S (%) | $BD_R/BD_S$ |
| Kassi | 0.391 | 0.100 | 4.71 | 1.16 | 72.2 | 0.93 |
| Väljaküla | 0.474 | 0.102 | 2.96 | 1.11 | 57.8 | 0.89 |
| Lutsu | 0.409 | 0.115 | 2.70 | 1.10 | 45.0 | 0.95 |
| Kambja | 0.651 | 0.114 | 1.70 | 1.06 | 65.1 | 0.98 |
| Haaslava | 0.633 | 0.127 | 1.80 | 1.05 | 65.2 | 0.90 |
| Pühatu | 1.103 | 0.221 | 1.42 | 1.07 | 44.5 | 0.93 |
| Reigi | 1.511 | 0.330 | 1.65 | 1.04 | 71.2 | 1.01 |

indicates rhizosphere effect on bacterial activity, decreased with increasing bulk soil pH ($r^2 = 0.82$, $P < 0.05$; Fig. 4); i.e. stands with higher soil pH had a relatively smaller difference in the metabolic activity of bacteria between rhizosphere and bulk soil. When bulk soil pH was 5 or higher, the ratio $SA_R/SA_S$ did not decrease significantly ($P > 0.05$) any more. The highest $SA_R/SA_S$ value was revealed in the most acidic Kassi soil, and smallest values were revealed in soils with the highest pH (Reigi and Pühatu). Summed activity ratio $SA_R/SA_S$ correlated positively with birch short-root N % ($r^2 = 0.71$; $P < 0.05$, Fig. 5). The association between $MD_R/MD_S$ and soil pH was negative ($r^2 = 0.65$; $P < 0.05$; Fig. 4), but the slope was very small. Metabolic diversity of bulk soil was higher by higher soil pH.

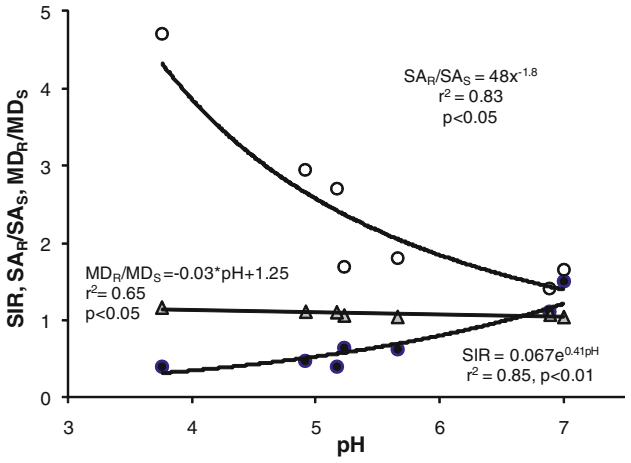


**Fig. 4** Microbiological characteristics: *SIR* (mg C $g^{-1}$), summed activity ratio $SA_R/SA_S$ and metabolic diversity ratio ($MD_R/MD_S$) depending on bulk soil

Bulk soil SIR and BAS (Table 4) were in a strong correlation ($r^2 = 0.98$; $P < 0.001$). SIR increased exponentially with bulk soil pH ($r^2 = 0.85$; $P < 0.01$; Fig. 4), linearly with soil N %, ($r = 0.94$; $P < 0.01$), and decreased linearly with increasing soil C/N ratio ($r = 0.85$; $P < 0.05$). The highest SIR and BAS values were found in Reigi soil, where the soil pH, organic content and N % were the highest; the smallest values were in the most acidic Kassi soil (Table 4). The mean (±SE) metabolic quotient of bulk soil of our stands was 0.221 ± 0.014 mg $CO_2$ mg $C_{mic}^{-1}$ 24 $h^{-1}$; metabolic quotient varied between 0.175 and 0.282.

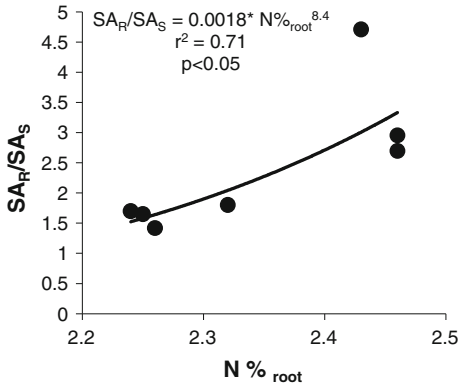


**Fig. 5** Relationship between summed activity ratio $SA_R/SA_S$ and short-root *N* concentration ($N\%_{root}$)

Hence, among measured soil chemical parameters, soil pH had the largest effect on the summed activity and metabolic diversity of soil microbial communities. Soil N correlated with BAS ($r = 0.90$; $P < 0.05$) and SIR only. Mean short-root mass was in a positive correlation with metabolic diversity in rhizosphere ($r = 0.78$; $P < 0.05$).

## Discussion

Stands growing on abandoned agricultural lands are an appropriate ecosystem for detecting between-site differences in fine-root morphology and rhizosphere effect because within-site spatial variability of soil properties is generally low. Characteristically to former agricultural fields of Northern countries, soil C/N ratio remained below 23 in all studied stands; hence, soil microbial growth was more C-limited than N-limited (Kaye and Hart 1997). Although the age of studied stands varied between 8 and 14 years, we considered that the effect of tree age on the results is negligible because most age-related changes in short-root morphology as well as rhizosphere effect of silver birch occur before age 5–7 years (Rosenvald et al. 2011, Rosenvald et al. unpublished data) and our stands were older.

### Morphological adaptations of short roots

Short-root morphology is tree species specific, as is the range of morphological adaptations in response to biotic and abiotic environmental conditions (Ostonen et al. 2007b). Compared to other tree species, silver birch is characterized by thin and densely branched roots that provide an efficient foraging system for nutrient uptake (Curt and Prevosto 2003; Ostonen et al. 2007b).

Mean short-root SRL in studied stands (60–76 $mg^{-1}$), except in Reigi, was in a similar range with SRL of 6- to 15-year-old birches growing in natural stands in fertile Oxalis forest site type (64–68 $mg^{-1}$, unpublished data). SRL of silver birches in Reigi was 1.4–1.7 times higher than in other stands. Higher root SRL leads to better exploitation of soil by roots, facilitating nutrient uptake (Richardson et al. 2009). Mean (±SE) short-root SRL of birches in Reigi ($107 \pm 6$ $mg^{-1}$) resembles SRL of young (5-year-old) birches growing in afforested oil shale mine areas ($112 \pm 9$ $mg^{-1}$; Rosenvald et al. 2011). High soil pH (≥7), water deficiency and planted (not naturally regenerated) trees are characteristic of both stands. P was not limiting in all stands, but leaf N and K of birches growing in Reigi remained under optimal ranges for birches (25–40 and 10–15 mg/g, respectively) according to Oleksyn et al. (2000). There are many reasons why it is difficult for plants to access nutrients in Reigi: (1) the low percentage (<20%) of fine earth in soil (Rendzic Leptosol) profile, which affects water-holding capacity and soil NPK pools negatively, (2) the summer drought as well as smaller annual precipitation than in other sites and (3) high bulk soil pH limiting nutrient, in particular phosphorus uptake. Under growth-limiting conditions, SRL should be minimized to enable a large soil volume to be exploited with a small root construction cost (Ryser 2006). Also in our study, birch short-root SRL and SRA correlated negatively with leaf N %. We suggest that high short-root SRL and SRA may compensate for low nutrient supply rates and could be used as stress indicators in young silver birch stands on abandoned agricultural land.

Besides SRL, short-root ramification is another important root characteristic affecting root nutrition. Higher short-root ramification favours P and N uptake (Richardson et al. 2009) and leads to a higher proportion of subapical parts or of first-order roots, which release exudates more than maturer root regions (Jones et al. 2009). Under heterogeneous soil conditions, root branching is highly sensitive to environmental stimuli, particularly nutrient availability (Hodge et al. 2009). Also in our study, the most sensitive morphological parameter responding to environmental conditions was short-root ramification (number of tips per mass unit).

The structure of the EcM fungal community affects morphology and functioning of ectomycorrhizal short roots of trees (Ostonen et al. 2009; van der Heijden and Kuyper 2003). Both short-root L and D are strongly influenced by the EcM fungus (Ostonen et al. 2009). As short-root L and D had a high correlation with the second axis in the PCA analysis in our study, the variation along the second axis could most probably be explained by the species-specific impact of the EcM fungal partner. The first axis captured mass-related parameters and, of the supplementary variables, leaf N.

Rhizosphere effect

In addition to the morphological adaptation of roots, using rhizodeposition to support rhizosphere bacteria is another strategy of trees to improve mineral nutrition (Lõhmus et al. 2007). In this strategy, root exudation is a key process for carbon transfer into the rhizosphere (Singh et al. 2004). Root exudation and other rhizodeposition components cause the rhizosphere effect, which we measured as the difference or ratio between soil and rhizosphere characteristics.

Bacterial activity in rhizosphere was 1.4–4.7 times higher than in bulk soil, which indicates great between-site differences in root support to the rhizosphere bacterial community. A similar result was found for young silver birches in reclaimed oil shale mining areas and for alder stands in different habitats where rhizosphere SA was even up to two orders of magnitude higher than SA of bulk soil (Lõhmus et al. 2006a, b; Lõhmus et al. 2007). Similar to the bacterial activity (SA), metabolic diversity was also higher in rhizosphere than in bulk soil in studied birch stands because the bacterial communities in rhizosphere are adapted to use root exudates as key carbon sources; in bulk soil, the role of root exudates is essentially smaller. Adaptation of rhizosphere bacterial communities to root exudates is most probably also the reason for three times higher between-stands variation of SA in bulk soil than in rhizosphere.

Broad soil pH gradient affected the activity and diversity of soil microbial communities the most, e.g. by causing great differences between stands in bulk soil SIR (about 4 times) and BAS (about 3 times). The mean (±SE) of metabolic quotient of bulk soil measured in our sites ($0.22 \pm 0.01$) was low like elsewhere on abandoned agricultural lands in Estonia ($0.26 \pm 0.02$; Truu 2008). The low values of metabolic quotient in soils of afforested agricultural lands indicate that the soils have acquired the structure and properties providing conditions for better stabilized microbial communities (dominated by K-strategic species characterized by a slow growth rate and stable biomass development). In our study, soil pH correlated positively with bulk soil SA and even with metabolic diversity of bulk soil. This also corresponds to the results of White et al. (2005), which showed that values of substrate utilization were higher in soils of higher pH.

Bacterial community diversity was higher in bulk soil than in rhizosphere (except Reigi), indicating that bacterial community structure was shaped by rhizodeposition. The large effect of rhizodeposition on bacterial community structure was also seen in the low similarity between DGGE profiles of rhizosphere and bulk soil. In our study, bacterial community diversity did not correlate with bulk soil pH. Fierer and Jackson (2006) studied a wide range of ecosystem categories from tundra to tropics and found the highest bacterial diversity in neutral (pH around 7.0) soils. However, in boreal forest/tundra ($4 < \text{pH} < 6$), no effect of soil pH on bacterial diversity was revealed, which corresponds to our result.

Root-induced changes in rhizosphere pH play a major role in the bioavailability of the many pH-dependent nutrients (Hinsinger et al. 2006). In stands with high soil pH, trees must acidify the rhizosphere in order to receive nutrients that are soluble in an acid environment such as P and Fe (Marschner 2005). Trees growing on soil with low pH increase rhizosphere pH in order to make the rhizosphere suitable for microbes and to make major nutrients such as K, Ca, Mg, P, S and Mo available for uptake by plant roots (Dakora and Philips 2002). According to our data, rhizosphere processes do not change the rhizosphere pH if stand bulk soil pH nears 4.3. It is not excluded that in further studies the absolute value of pH limit may be different from 4.3 as only seven stands were investigated in our study. However, due to the broad pH gradient of the stands involved in our study, the pH threshold most probably remains between pH values 4 and 5. The greatest decrease in rhizosphere pH occurred in Pühatu and Reigi stands, which had the highest bulk soil pH. However, the summed activity ratio $SA_R/SA_S$ was small there. Therefore, it is likely that acidification of the rhizosphere occurs in these stands as a release of $H^+$ without a significant loss of C from roots (Marschner 2005).

A negative relationship between bulk soil pH and summed activity ratio $SA_R/SA_S$ indicates that in the case of lower soil pH, birch supports more rhizosphere bacteria and the rhizosphere effect is more pronounced. Phillips and Fahey (2006) found that rhizosphere effect on net N mineralization and phosphatase activity was much greater in soils with $pH_{H_2O} < 4.3$ for EcM tree species and suggested that soil pH and its relation to nutrient availability may also influence the magnitude of rhizosphere effects. However, in our study, when bulk soil pH was ≥5, the decrease in $SA_R/SA_S$ was insignificant, i.e. the rhizosphere effect stayed at a stable level.

Very low variability of short-root N % compared to three times higher variability of leaf N % between stands shows the stable nitrogen status of short roots, which is little dependent on soil properties. Short roots are first served by assimilated nutrients. Our mean short-root N % of silver birch ($2.35 \pm 0.38$) was significantly higher than short-root N % of deciduous *Quercus robur* L. ($1.97 \pm 0.29$) reported in Trocha et al. 2010. Root N concentration of deciduous trees is positively related to both respiration of the root (Makita et al. 2009; Pregitzer et al. 1998; Reich et al. 1998) and nutrient uptake rate (Reich et al. 1998), thus to the general physiological activity. Our study showed that short-root N concentration correlated positively with summed activity ratio $SA_R/SA_S$, indicating root support to rhizosphere bacteria; most probably, there is a positive feedback.

The similarity in bacterial community between rhizosphere and bulk soil was the greatest for Kassi, where bulk soil pH was the lowest and the difference between rhizosphere and bulk soil pH was the smallest. An unexpected finding was that the comparatively high similarity in bacterial communities between bulk soil and rhizosphere was found in Reigi, where rhizosphere and bulk soil pH differed by 1 pH unit. However, soil conditions in Reigi were less favourable due to the highest bulk soil pH, drought-susceptible Rendzic Leptosol and small local precipitation. The hypothesis whether there is a general tendency of higher similarity of bacterial communities between rhizosphere and bulk soil in harsh soil conditions needs further investigation.

The link between short-root morphological adaptations and rhizosphere effect was not fully revealed in the present study. Although Lõhmus et al. (2006a) found positive correlations for alders between short-root SRA and two microbiological characteristics—summed activity ratio $SA_R/SA_B$ and metabolic diversity ratio $MD_R/MD_S$—we did not find the same kind of correlation in the studied birch stands. Hence, tree species affects rhizosphere processes. Also, leaf NPK nutrition was not related to soil and rhizosphere bacterial characteristics. One possible reason could be that bacteria are more generalists. The soil fungi tend to be the primary saprotrophs in forest soils and also of considerable importance in regulating tree nutrient uptake. Because of differences in nutrient uptake capacities of individual fungal species (Courty et al. 2010) and their significant impact on root tip morphology (Ostonen et al. 2009), a shift in EcM colonizers community may result in significant changes in mineral nutrition. To find the link between tree nutritional status and mycorrhizal fungi communities, further studies are needed.

## Conclusion

Short-root morphological adaptations in young silver birch stands on abandoned agricultural land were affected by N-nutrition—functional short-root parameters (SRA, SRL) were higher by lower leaf N. The support provided by silver birch to rhizosphere bacterial communities correlated negatively with soil pH and positively with short-root N %. Rhizosphere pH increased with increasing bulk soil pH. Rhizosphere acidification occurred in studied stands when bulk soil pH $\geq$ 5. All studied microbiological activity and diversity parameters indicating rhizosphere effect were strongly affected by soil pH. The results show that strategies improving root nutrition—morphological adaptation and rhizosphere effect—depended on different environmental factors, which means they are based on different regulatory mechanisms. Our study increases the understanding about the factors controlling mineral nutrition of young silver birches; the approach can be applied to other tree species as well.

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