Rhizospheric effect and fine-root morphological adaptations in a chronosequence of silver birch stands on reclaimed oil shale post-mining areas

Katrin Rosenvald a, *, Tatjana Kuznetsova b, Ivika Ostonen a, Marika Truu a, Jaak Truu c, Veiko Uri b, Krista Lõhmus a

a Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia
b Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi 5, 51014 Tartu, Estonia
c Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51014 Tartu, Estonia

ARTICLE INFO

Article history:
Received 10 November 2009
Received in revised form 21 May 2010
Accepted 23 May 2010
Available online 3 July 2010

Keywords:
Betula pendula
Microbial activity
Bacterial species diversity
Fine-root morphology
Mineral nutrition
Post-mining reclamation

ABSTRACT

Mining activities create wastelands that require reclamation. The relief of abandoned opencast oil shale mining area is rugged, and the mining spoil is extremely stony and alkaline (pH 8), with low N and organic content. Planting of fast-growing deciduous tree species such as silver birch (Betula pendula) on post-mining area is the best means to accelerate the development of a new forest ecosystem in such harsh conditions. A chronosequence of silver birch stands (1, 2, 3, 5, 29, 40 years old) was investigated to reveal changes in bulk soil (S) and rhizosphere (R) properties, in rhizosphere effect on bacterial activity and diversity, and in fine-root morphological adaptations in relation to stand development. The rhizosphere effect on bacterial activity was measured as a rhizosphere/soil (R/S) ratio and on species diversity as a similarity (%) between rhizosphere and bulk soil bacterial communities. Bacterial species diversity was determined by denaturing gradient gel electrophoresis (DGGE) technique and was expressed as Shannon diversity index. Biolog EcoPlates were used to determine the summed activity of cultivable bacteria in rhizosphere and bulk soil. Short-root morphological parameters were measured using WinRHIZOTM Pro. Soil pH and available P concentration decreased logarithmically, and N% and organic matter concentration increased linearly with increasing stand age. During the first 30 years of stand development SIR increased an order, from 0.18 to 1.90 mg C g−1. Bulk soil bacterial diversity increased logarithmically with stand age. The bacterial diversity was highest in rhizosphere than in bulk soil. Rhizosphere effect on bacterial activity was low 1 year after planting, increased more than two times in the next 2 years, and decreased thereafter rapidly with stand age. Rhizosphere effect, indicating plant support to rhizosphere microbial communities, was highest when soil conditions were still poor, but trees had already overcome the transplant shock. All short-root morphological parameters showed certain trends with age. Specific short-root length varied between 56 and 313 mg−1 and decreased logarithmically with stand age and soil improvement. The fastest changes in short-root morphology, rhizosphere effect, and soil pH occurred during the early development of silver birch stands – in the first 5 years; P nutrition and N use efficiency improved simultaneously. Rhizosphere effect and short-root morphological adaptation have an important role in soil and stand development on oil shale post-mining area, and silver birch is a promising tree species for reclamation of alkaline mining spoil.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Opencast oil shale mining creates alkaline (pH ~ 8) wasteland that requires reclamation. After opencast mining, the relief is rugged and the soil heterogeneous and extremely stony; N and organic content of oil shale mining spoil is low. Planting of trees, especially fast-growing deciduous species such as silver birch on abandoned opencast oil shale mining areas is the best means to accelerate the development of a new forest ecosystem in such harsh conditions. In Estonia, forest rehabilitation of skeletal calcareous detritus of opencast oil shale mining has been carried out since 1960 in an area of over 10,000 ha.

Soil formation and the development of the whole ecosystem on levelled spoil of post-mining areas can be considered as a primary succession, which can be accelerated by planting trees. In comparison with unplanted sites, tree plantations have a marked catalytic effect on forest and soil development in severely degraded sites (Filcheva et al., 2000; Parrotta et al., 1997; Pietrzykowski

* Corresponding author.
E-mail address: Katrin.Rosenvald@ut.ee (K. Rosenvald).

0925-8574/5 – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ecoleng.2010.05.011
and Krzaklewski, 2007; Wang et al., 2007). Forest trees, which are dominant plants, act as an environmental filter that controls the availability of resources and the composition of understory vegetation (Pensa et al., 2008). Tree plantations can be used as a tool for mine spoil restoration since they have the ability to restore soil fertility and ameliorate microclimatic conditions (Singh et al., 2002). Interactions between plants and microorganisms seem to be of great importance for elemental transfer towards the plant and the soil. These interactions heavily control soil characteristics (Dilly et al., 2000).

Soil bacterial biomass and activity increase simultaneously during soil development on mining spoil (Banning et al., 2008; Chodak et al., 2009; Insam and Domsch, 1988; Kristufek et al., 2005; Lõhmus et al., 2006a; Šourková et al., 2005a). High microbial activity has in turn a positive effect on soil quality. Information about the succession of soil and rhizosphere bacterial communities in developing forests on mining spoil is scarce. Rhizodeposition supports higher microbial biomass and microbial activity in the rhizosphere and, although rhizodeposition strongly affects the structure and activity of soil microbial communities and plant nutrition, few studies focus on the root surface bacteria of forest trees. The interaction between soil microbial communities and roots should be particularly important in harsh site conditions, including reclaimed opencast oil shale mining areas (Lõhmus et al., 2006a; Walker et al., 2004).

Simultaneously with the rhizosphere effect, morphological adaptations of fine roots directly affect the nutrition of trees. Morphological plasticity of fine roots has been proposed as a mechanism by which plants respond to variation in soil nutrient supply (Hodge, 2004; Lõhmus et al., 1989; Ostonen et al., 1999). Alterations in fine-root morphological traits reflect exploitation of water and nutrients in the soil (Fitter, 1996) as well as the cost/benefit ratio of the fine-root system (Ostonen et al., 2007). Most frequently measured functionally important morphological features of fine roots are specific root length, specific root area, and root tissue density which are believed to be indicative of environmental changes. Hence, both rhizosphere processes – fine-root morphological adaptations and the shaping of microbial communities in rhizosphere and soil – are important strategies to ensure the sustainability of developing forest ecosystems on oil shale post-mining areas (Lõhmus et al., 2006a,b, 2007).

The choice of tree species is a decisive factor in the success of mine reclamation. Deciduous trees, including silver birch, have a number of advantages: the increased N and P availability in the soil, faster growth at a young age, and higher resistance to pests, diseases, and fires in comparison with conifer monocultures (Lõhmus et al., 2007). Silver birches (Betula pendula Roth.), similarly to other pioneer species, perform an important function in ecosystems – soil improvement. Silver birch is economically the most important indigenous deciduous tree in the boreal and partly in the temperate zone of the Northern hemisphere. Silver birch (B. pendula) and black alder (Alnus glutinosa) are the most successful native deciduous trees on reclaimed opencast oil shale mines (Lõhmus et al., 2007). This is why the potential of silver birch for the reclamation of exhausted opencast oil shale mines requires thorough investigation.

Rhizosphere processes are of central importance concerning terrestrial ecosystem functioning (Hinsinger et al., 2009). Their role in forest succession on reclaimed post-mining areas is crucial but still poorly understood. There are few data about the rhizosphere effect on microbial communities and about changes in root morphology in relation to soil formation and plantation stand age. Ostonen et al. (2006) found that stand age had a significant impact on short-root parameters of black alder growing on oil shale mining area. In the 1-, 2-, and 27-year-old silver birch and black alder stands growing on a reclaimed oil shale mining area, the greatest difference between the activity of the rhizosphere and bulk soil microbial communities was observed in the second year after planting, when the surviving plants had overcome the transplant shock, the intensive strategy prevailed, and plants strongly supported rhizosphere microbial communities (Lõhmus et al., 2007). We used a chronosequence of 6 silver birch stands growing on oil shale post-mining areas to investigate the changes in (1) bulk soil microbial and chemical properties, (2) rhizosphere effect on bacterial activity and species diversity, and (3) fine-root morphological adaptations during soil formation after reclamation.

This paper aims to elucidate the role of rhizosphere effect and fine-root morphological adaptations, interacting with soil formation, in ensuring the mineral nutrition of trees in silver birch stands on reclaimed exhausted opencast oil shale mines.

2. Materials and methods

2.1. Site description

Opencast oil shale mining areas (see map in Lõhmus et al., 2007) were reclaimed with silver birch in 1967 (Aīdu), 1978 (Sirgala), 2002 (Narva I), and 2005 (Narva II). Two stands (Narva I and Narva II) were studied repeatedly at different stand ages. The first years of stand development are most critical for tree survival (Lõhmus et al., 2007; Kuznetsova et al., submitted for publication); therefore, a smaller age interval has been used at the beginning of the chronosequence – stands at the age of 1, 2, 3, 5, 29, and 40 years were included in the study (Table 1). Skeletal calcareous detritus of oil shale mining spoil contains 60–70% Ordovician ordinary and dolomitized limestones, making mining spoil highly coarse and calcareous (Reintam et al., 2002). Two-year-old bare-root seedlings were planted in all cases, except in 2005, when 1-year-old seedlings were used. Planting density was 2 m × 2 m in all stands. In two older stands Calcaric Regosol had already been formed. Volume per ha of 29- and 40-year-old stands is 179 and 303 m³ ha⁻¹, respectively.

2.2. Sampling

Soil and rhizosphere bacterial activity and short-root morphology were assessed in 2006 in all stands (stand ages: 2, 5, 29, and 40 years), in 2005 in Narva II (1 year) and in 2004 in Narva I (3 years). Bacterial community diversity by DGGE was assessed only in 2006. All samples were taken in October. Ten samples from the topmost 10 cm detritus of soil layer (20 cm × 20 cm) were collected randomly in a stand. A random fine-root 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). The method is thoroughly described in Lõhmus et al. (2006b). All roots were carefully removed by hand from the field-moist mineral soil which was then passed through a 2-mm mesh sieve to give the bulk soil (S) fraction. Both the dead and coarse roots (∅ ≥ 2 mm in diameter) were separated; the remaining fine roots (∅ ≤ 2 mm) and soil were carefully manually shaken 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 (R).

2.3. Microbiological methods

Substrate-induced respiration (SIR) by Isrømeyer technique was applied to measure metabolically active microbial biomass carbon. Glucose (0.4 g × 100 g⁻¹ 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 CO₂ 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 CO₂ was absorbed in 0.05 M sodium hydroxide, quantified by titration, and the respiration rate was calculated. The metabolic quotient (q(CO₂) = BAS SIR⁻¹) was also calculated.

Biolog EcoPlates method was used to determine the summed activities of bacterial communities in soil (SA₆) and in rhizosphere (SA₆); the method is thoroughly described in Truu et al. (2009). Higher ratio (SAR/SAS) is considered to indicate higher rhizosphere effect and greater plant support to the rhizosphere microbial communities.

Bacterial community diversity, expressed as Shannon diversity index, was measured for bulk soil (SD₆) and for rhizosphere (SD₆) using DNA based denaturing gradient gel electrophoresis (DGGE) technique thoroughly described in Truu et al. (2009). The rhizosphere effect on bacterial community diversity was calculated as the similarity in bacterial community between rhizosphere and bulk soil and was expressed as Pearson’s correlation coefficient multiplied by 100%.

2.4. Short-root morphological parameters

Short roots (see definition by Ostonen et al., 2007) were used to analyse fine-root morphological adaptations. The most important differences in fine-root morphology are manifested in the first order roots, indicating that the first order roots may be a unique unit of fine-root system in understanding both the function and the dynamics of root systems in forests (Wang et al., 2006). In our study, only first and second order roots with primary structure were represented. This fine-root compartment is functionally homogeneous and most active in water and nutrient uptake (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 living short-root subsamples were taken per sample and root tips were counted under a microscope; there were 11–22 root tips in a subsample and 236–400 tips per stand in total. Short roots were considered living if the exposed stele was still shiny and resilient (Vogt and Persson, 1991). All studied short roots were ectomycorrhizal according to macroscopic features.

Short-root length, projection area, and mean diameter (D) of the sample were measured using WinRHIZOTM Pro 2003b (Regent Instruments Inc.). After measuring, short-root samples were dried at 70 °C for 2 h (enough to get constant weight) and weighed to an accuracy of 0.01 mg. The method for determining short-root morphological parameters (mean short-root length (L; mm), specific root area (SRA; m² kg⁻¹), specific root length (SRL; m g⁻¹), tissue density (RTD; kg m⁻³), root tip frequency per 1 mg dry mass (RTFM; mg⁻¹), and per 1 mm root length (RTFL; mm⁻¹)) is given in detail in Ostonen et al. (2007).

2.5. Chemical analysis

Soil nitrogen was determined according to Kjeldahl; Tecator ASN 3313 was applied. Determination of available (ammonium lactate extractable) phosphorus in the soil was performed by flow injection analysis, with the use of Tecator ASTN 9/84. Ash content and loss on ignition (LOI) were determined at 550 and 360 °C, respectively. pHKCl of samples was measured for both bulk soil as well as rhizosphere. Leaf NPK was measured in 2002–2004. Leaf samples were analysed for total Kjeldahl nitrogen, total Kjeldahl phosphorus, and potassium concentration.

The total N, C, and H concentrations in short roots were determined using CHN analyser PerkinElmer 6400 Series II in the laboratory of the Department of Geology of the University of Tartu.

2.6. Statistical methods

Normality of variables was checked by Lilliefors and Shapiro–Wilk’s tests. When necessary, log- and root-transformations were used to normalize the data. Differences between stand means of short-root characteristics were checked by Tukey’s test for unequal n. The homogeneity of the group variances was checked by Levene’s test; the group variances were homogeneous for SRA, L, and W. Principal component analysis (PCA) was applied for analysing short-root morphological parameters; soil pH, P, K concentrations were used as supplementary variables. Level of significance a = 0.05 was accepted in all cases. Software STATISTICA 7.0 was used.

### Table 1

Characteristics of silver birch stand chronosequence growing on reclaimed oil shale mining area: trees/ha, number of trees/ha; DBH, mean diameter at breast height.

<table>
<thead>
<tr>
<th>Age of stand</th>
<th>Stand</th>
<th>Stand characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trees/ha</td>
<td>Mean height (m)</td>
</tr>
<tr>
<td>1</td>
<td>Narva II</td>
<td>1600</td>
</tr>
<tr>
<td>2</td>
<td>Narva II</td>
<td>1540</td>
</tr>
<tr>
<td>3</td>
<td>Narva I</td>
<td>1170</td>
</tr>
<tr>
<td>5</td>
<td>Narva I</td>
<td>1040</td>
</tr>
<tr>
<td>29</td>
<td>Sigrala</td>
<td>1660</td>
</tr>
<tr>
<td>40</td>
<td>Aidu</td>
<td>1490</td>
</tr>
</tbody>
</table>

* Diameter at root collar.

### Table 2

Soil characteristics in 0–10 cm layer: nitrogen concentration (N), and loss on ignition (LOI%), substrate-induced respiration (SIR), basal respiration (BAS), and metabolic quotient (Q); DGGE data: Shannon diversity index of rhizosphere (SD₆) and bulk soil (SD₆) bacterial community, and similarity between rhizosphere and bulk soil bacterial communities (RS similarity).

<table>
<thead>
<tr>
<th>Stand age (years)</th>
<th>Soil characteristics</th>
<th>DGGE data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>LOI (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.031</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>0.023</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>0.039</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>0.050</td>
<td>4.2</td>
</tr>
<tr>
<td>29</td>
<td>0.325</td>
<td>14.9</td>
</tr>
<tr>
<td>40</td>
<td>0.329</td>
<td>14.7</td>
</tr>
</tbody>
</table>

n.e., not estimated.
3. Results

3.1. Dynamics of bulk soil characteristics

Initial N and organic matter content of mining spoil are low (Table 2), and in young stands a significant proportion of the organic matter consists of oil shale mining residues (Reintam et al., 2002). Soil organic matter (LOI) and N increased with stand age ($r = 0.98$, $p < 0.01$ for both). Available soil P was initially quite high (near 80 mg kg$^{-1}$) and decreased with stand age and soil development. Both soil pH and P concentrations decreased logarithmically with stand age ($r = 0.93$ and $r = 0.92$, respectively, $p < 0.01$ for both; Fig. 1a and b). Initially low nitrogen and organic matter (estimated by LOI) content are in accordance with low microbial biomass indicated by SIR (Tables 2 and 3). SIR and BAS correlated both positively with organic matter and soil N% ($0.86 < r < 0.97$, $0.01 < p < 0.05$). During the first 30 years of stand development, SIR increased by approximately one order of magnitude (Table 2).

3.2. Activity and diversity of bacterial communities in bulk soil and rhizosphere

Rhizosphere pH was lower than bulk soil pH in all studied stands. The diversity of bacterial communities (SD) in studied stands was higher in rhizosphere than in bulk soil (Table 2); the difference between diversity indexes (SD$_B$−SD$_R$) decreased linearly with increasing stand age ($r = −0.96$). According to this relationship, SD$_B = SD_R$ at the stand age of 53 years; after that the diversity of the bacterial community will be higher in bulk soil than in rhizosphere. The similarity between bacterial communities in rhizosphere and bulk soil was low, remaining in the range of 33–42% (Table 2). According to the dendrogram based on DGGE analysis, two groups were formed. The first group included rhizosphere and bulk soil of younger stands (2 and 5 years old) (Fig. 2), and the second group included older stands (29- and 40-year-old stands in Sirgala and Aida, respectively) on already formed soil (Rendzic Leptosol). The only exception was the rhizosphere of the 2-year-old stand in the second group; this rhizosphere was still affected by the soil that had adhered to the roots in the nursery.

Diversity of bulk soil bacterial community increased linearly with decreasing soil pH ($r = 0.97$, $p < 0.05$) and increased logarithmically with stand age (SD$_B = 0.13 \ln(A) + 3.2$; $r = 0.97$, $p < 0.05$) as well as soil N% (SD$_B = 0.16 \ln(N\%) + 3.9$; $r = 0.95$, $p < 0.05$). The rhizosphere effect on summed bacterial activity (SA$_B$/SA$_R$), indicating plant support to rhizosphere microbial communities, was low a year after planting, increased more than two times in the next 2 years, and decreased thereafter hyperbolically with age as shown in Fig. 3.
Table 3
Mean short-root morphological parameters (±standard errors): D, diameter; RTD, root tissue density; RTFM, root tip frequency per 1 mg dry mass; RTE, root tip frequency per 1 mm root length; short-root C and N concentrations and C/N ratio.

<table>
<thead>
<tr>
<th>Stand age</th>
<th>Mean D (mm)</th>
<th>RTD (kg/m³)</th>
<th>RTFM (1/mg)</th>
<th>RTE (1/cm)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.282 ± 0.009</td>
<td>540 ± 5 a</td>
<td>127 ± 17ab</td>
<td>4.06 ± 0.42 a</td>
<td>42.2</td>
<td>2.31</td>
<td>18.3</td>
</tr>
<tr>
<td>2</td>
<td>0.275 ± 0.006</td>
<td>88.4 ± 5 a</td>
<td>107 ± 9d</td>
<td>5.37 ± 0.30 ab</td>
<td>41.6</td>
<td>2.24</td>
<td>18.6</td>
</tr>
<tr>
<td>3</td>
<td>0.319 ± 0.100</td>
<td>67.7 ± 3 a</td>
<td>123 ± 10d</td>
<td>6.48 ± 0.41 b</td>
<td>39.8</td>
<td>2.23</td>
<td>17.8</td>
</tr>
<tr>
<td>5</td>
<td>0.281 ± 0.009</td>
<td>154 ± 3d</td>
<td>76.6 ± 6c</td>
<td>6.90 ± 0.26 b</td>
<td>42.3</td>
<td>2.05</td>
<td>20.6</td>
</tr>
<tr>
<td>29</td>
<td>0.374 ± 0.006</td>
<td>167 ± 4d</td>
<td>50.3 ± 2a</td>
<td>9.07 ± 0.33 c</td>
<td>43.7</td>
<td>2.35</td>
<td>18.6</td>
</tr>
<tr>
<td>40</td>
<td>0.367 ± 0.000</td>
<td>145 ± 4c</td>
<td>65.4 ± 4b</td>
<td>9.66 ± 0.36 b</td>
<td>43.9</td>
<td>2.40</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Different letters denote significant differences between stands (Tukey’s test; P < 0.05).

Fig. 4. (a) Short-root mean length (L) and (b) specific root area (SRL) in silver birch stands of different ages; A, stand age.

(D) increased linearly with stand age (r = 0.91, p < 0.05); mean short-root tip frequency per root length (RTFL = 1.4 Ln(A) + 4.4; r = 0.99, p < 0.01) and tissue density (RTD = 27 Ln(A) + 62; r = 0.84, p < 0.05) increased logarithmically with stand age. Mean short-root length (L; Fig. 4a), specific length (SRL; Fig. 4b), and tip frequency per root mass (RTFM = −19 Ln(A) + 125; r = 0.90, p < 0.05) decreased with stand age. Mean specific root area (SRA) correlated highly with specific root length (r = 0.99, p < 0.01) and decreased from 274 to 65 m² kg⁻¹ with stand age according to the equation SRA = 239A⁻0.36 (r = 0.93, p < 0.01).

The two first factors of PCA analysis accounted for the highest percentage of the variation in short-root morphology (98%) when diameter, tissue density, and specific root length were included (Fig. 5). The first axis accounted for 77% and the second axis for 21% of the variation in short-root morphology (Fig. 5a). N and available P concentrations as well as soil pH were used as supplementary variables. A strong correlation with the first axis was found for short-root SRL (r = −0.98, p < 0.01). Also soil characteristics (N, available P, and pH) correlated highly with the first PCA axis. The second PCA axis correlated best with short-root D (r = −0.62, p < 0.01). In the PCA ordination plot (Fig. 5b), the stands were ordered along the first axis by stand age, whereas the two older stands formed one group. Hence, the fastest changes in short-root morphology of silver birch occurred at a young age.

All morphological parameters included into PCA were in a significant correlation with soil P concentration and pH (0.85 ≤ |r| ≤ 0.93, 0.01 < p < 0.05). Short-root D and SRL correlated both with log soil N% (r = 0.94, p < 0.01 and r = −0.81, p < 0.05, respectively).

Short-root SRL, L, and SRA were in a negative correlation with bacterial community diversity (Shannon diversity index) of rhizosphere and bulk soil (−0.99 < r < −0.95, p < 0.05). When we excluded the first year data, influenced by the transplant shock, all measured short-root parameters, excluding D, were correlated with the rhizosphere effect on summed activity – SAR/SAS (0.89 ≤ |r| ≤ 0.99, 0.01 < p < 0.05).

Fig. 5. (a) Principal component analysis based on correlation matrix of short-root morphological parameters. Abbreviations: SRL, specific root length; RTD, root tissue density; D, short-root diameter. Supplementary variables (indicated by asterisks): soil pH, soil N, and P. (b) Ordination of stands of a chronosequence by short-root morphological parameters along the first two PCA axes (F1 × F2). Numbers indicate stand ages.
3.4. Short-root C and N concentration

Short-root C and N concentrations (Table 3) had low variability between stands of different age (coefficients of variation were 0.040 and 0.060, respectively). The highest short-root N and C concentrations were observed in the oldest (43-year-old) stand. Short-root C concentration was higher by lower short-root RTFM \( r = -0.94 \). Short-root C correlated also with other morphological parameters related to short-root mass (SRA, \( r = 0.93 \); SRL, \( r = -0.91 \); RTD, \( r = 0.88 \); \( p < 0.05 \) for all cases). Short-root C concentration was higher when rhizosphere effect, indicated by \( S_{AR}/S_{AS} \), was higher (\( r = 0.92, p < 0.05 \)).

4. Discussion

4.1. Soil formation and improved mineral nutrition

Biological reclamation with silver birch rapidly and significantly improved the quality of the growing substrate on oil shale post-mining areas. Accumulation of N and organic matter in the surface layers of the spoil material is crucially important for the soil formation process during primary succession after stand establishment. On reclaimed oil shale post-mining areas, soil N increased more than ten times and organic matter content almost four times during the first 40 years. These results are consistent with many other studies, which have shown a rapid increase in soil organic matter and recent carbon as well as N content on afforested post-mining areas (e.g. Banning et al., 2008; Karu et al., 2009; Pietrzykowski and Krzaklewski, 2007). Simultaneously with stand development, soil pH decreased logarithmically by approximately 1 pH unit during the first 30–40 years. Lower pH favours plant P uptake, and a production and mineral nutrition study carried out in the same stands (in Narva I, II) showed that leaf P% increased during the first 7 years from 0.19 ± 0.04 to 0.32 ± 0.04 (Kuznetsova et al., 2010, submitted for publication). Leaf P concentration was in the optimal range for birch (0.15–0.30%, Oleksyn et al., 2000), but leaf N% and K concentrations in young silver birch stands growing on reclaimed oil shale mining areas (Kuznetsova et al., submitted for publication) were above optimal ranges according to Oleksyn et al. (2000). However, the annual aboveground production per leaf N mass unit – nitrogen use efficiency – increased two times (40–80 year\(^{-1} \)) in a chronosequence of 2–7-year-old stands (Kuznetsova et al., submitted for publication).

The decrease in soil pH is caused not only by plant litter input and decomposition, but also by rhizodeposition, including acidic root exudates, because the acidifying effect of the rhizosphere (by up to 1.5 pH units) was revealed in all cases. A decrease in pH on alkaline post-mining areas in planted or spontaneously formed forests is a general rule (Baldrian et al., 2008; Frouz and Novakova, 2005; Sourková et al., 2005a). The loss in soil available P may relate to increasing uptake and assimilation of P by plants during stand development (Sourková et al., 2005b).

4.2. Dynamics of soil microbial activity and diversity

Despite the fact that microorganisms form only about 2–4% of the soil organic matter, their activity is one of the principal processes of soil formation through their high turnover rate and their role in organic matter transformation (Sourková et al., 2005a). Plants growing on nutrient-poor substrates are especially dependent on nutrients that are released from the decaying organic debris on the forest floor (Leuschner and Rode, 1999). Reclamation measures not only improve the gross microbial properties but also promote rapid development of metabolic abilities characteristic of natural forest soil microbial communities (Chodak et al., 2009). Oil shale mining spoil is initially extremely low in nitrogen and organic matter, which is why the microbial biomass (SIR) is initially very low but grows rapidly during the succession of the stand. This result is consistent with previous studies on mining areas (Banning et al., 2008; Chodak et al., 2009; Kristufek et al., 2005; Sourková et al., 2005b).

Many authors have reported a decrease in the metabolic quotient (indicating carbon availability in the soil) during the initial stages of succession on post-mining areas (Banning et al., 2008; Frouz and Novakova, 2005; Sourková et al., 2005b). However, in our study the metabolic quotient had no clear age-related tendency. Insam and Domsch (1988) reported a significant decrease in the metabolic quotient with time on agricultural sites but not on forest sites. The suitability of the metabolic quotient as a bioindicator of ecosystem development can be limited because it fails to distinguish between the effects of disturbance and stress (Wardle and Ghani, 1995). One reason for contradictory data concerning the metabolic quotient is that BAS responds rapidly to environmental fluctuations.

The rhizosphere effect on summed bacterial activity (\( S_{AR}/S_{AS} \)), indicating the support of rhizosphere microbial communities, had the highest values in the second and third year after planting, when surviving plants had overcome the transplant shock, and decreased rapidly after that. In the middle-aged stands, where soil conditions were greatly improved and \( \text{pH} \) had decreased, \( S_{AR}/S_{AS} \) was low, showing that the need for rhizosphere support was not as important there. These results are consistent with the findings by Lõhmus et al. (2007), who studied black alder and silver birch stands growing on an oil shale mining area. However only 1–4, and 27–year-old stands were involved in the study and early dynamics could not be followed properly. The authors raised the following question: why are rhizosphere communities poorly supported in the first year when the need is most urgent? The possible explanation was that during the first year the intensive strategy of the fine-root development prevailed in order to exploit the oil shale mining spoil by fine roots as much as possible, and there was a deficit in assimilates allocated below ground.

According to the dendrogram based on DGGE, the rhizosphere of 2-year-old birches belonged to the same group with the rhizosphere and bulk soil of 29–40-year-old stands. After planting, the microbial community in the rhizosphere is not adapted to the harsh conditions of mining spoil and is most probably affected by the community in the soil adhering to bare-root seedlings in the nursery. Hence DGGE analysis reflects the succession of bacterial communities, but it is also sensitive to introduced bacteria adhering to the root systems of transplants. The diversity of bacterial communities of bulk soil increased simultaneously with soil formation, at the same time the diversity of bacterial communities was always higher in rhizosphere (\( S_{DR} \)) than in bulk soil (\( S_{DB} \)). Such an increase in microbial community diversity probably reflects the diversification of soil conditions during the soil formation process. There are two main factors that contribute to this process – change in soil chemical conditions (a greater number of different organic substrates) and increased habitat heterogeneity. However, the difference between diversity indexes (\( S_{DR} \sim S_{DB} \)) decreased linearly with increasing stand age, and after the stand age of 53 years (when \( S_{DR} > S_{DB} \)), the diversity in rhizosphere should be continuously lower than in bulk soil. Lower diversity of bacterial communities in rhizosphere than in bulk soil is commonly found in several ecosystems (Berg and Smalla, 2009). The rhizosphere effect on shaping the structure of the rhizosphere bacterial communities was massive because the similarities between communities in rhizosphere and bulk soil were low, remaining in the range of 33–42% only. The greatest difference between rhizosphere and bulk soil bate-
rial communities was observed in the second year after planting, when plants strongly support rhizosphere microbial communities.

4.3. Dynamics of short-root adaptations

Few papers have studied fine-root morphological parameters during stand development. Dynamics of short-root morphology in a chronosequence of silver birch stands were affected simultaneously by tree age and improved mineral nutrition. The PCA analysis showed that soil properties (pH, N, and available P) are important factors determining short-root morphology.

Roots of fast-growing species generally have greater SRL (Comas and Eissenstat, 2004), hence short-root SRL can be considered as an indicator of fast growth. The highest SRL in young silver birch stands on reclaimed oil shale post-mining areas indicates intensive exploration of highly stony and alkaline mining spoil by fine roots. Our results are in accordance with findings of Claus and George (2005), where fine-root SRL of both Picea abies (L.) Karst. and Quercus cerris L. was significantly higher in very young stands compared to stands of older age classes. In our study, all short-root morphological parameters showed certain trends with age, which are most probably also related to improved soil condition in addition to increase in age. Comparable data on age-related changes in fine-root morphological parameters are scarce. Belja et al. (2008) found for a chronosequence of 10-, 30-, 60-, and 120-year-old Norway spruce stands that RFT_M (the finest root fraction (<1 mm) in the 10-year-old stand (4 tips g⁻¹) was two times higher than that of older stands (2 tips g⁻¹). In our study, RFT_M was also approximately two times higher for younger silver birches (1–3 years) than for older trees (29 and 40 years). The main reason why young silver birches have high short-root tip frequency per mass unit is the low tissue density, which is approximately three times smaller than that for middle-aged trees. The age had no effect on spruce SRL of the finest (<1 mm in diameter) root fraction, however, spruce stands younger than 10 years were not involved in the study. However, the fastest changes in short-root morphology of silver birch occurred during the early development of stands before 10 years of stand age.

In our study, stand development (older trees and higher soil N) led to thicker and heavier short roots, and, also less root tips per unit short-root mass were formed. Hence, it seems that finer short roots, higher SRL, and higher root tip frequency per mass unit are primarily necessary for survival in unfavourable environmental conditions. Differences in short-root morphology along stand succession may also be caused by shifts in ectomycorrhizal fungi colonising short roots. Gebhardt et al. (2007) found differences in ectomycorrhizal morphotypes’ diversity and evenness along the chronosequence of Quercus rubra, and Ostonen et al. (2009) showed the impact of ectomycorrhizal fungal species on short-root traits of Alnus incana and A. glutinosa.

Short-root morphological parameters were related to the range of rhizosphere effect on bacterial activity. Most probably these correlations are affected by age because both morphological adaptations and rhizosphere effect have trends with age.

The chemical composition of short roots was stable in the silver birch chronosequence. The mean short-root N (2.14 ± 0.09) and C/N ratio (19.2 ± 1.4) of our 3- and 5-year-old silver birches were comparable with the ectomycorrhizal short-root N% (1.97 ± 0.29) and the C/N ratio (23.0 ± 1.8) of 4-year-old Quercus robur (Trocha et al., 2010). In our chronosequence, short-root N was lowest and C/N ratio was highest in the 5-year-old birch stand. Lower short-root N% and higher C/N ratio may indicate lower respiration and longer life span of roots (Trocha et al., 2010; Withington et al., 2006) as well as a shift in community composition of colonising ectomycorrhizal fungi. Hence, complex interactions between soil, rhizosphere, and fine-root morphology in developing silver birch stands were found on reclaimed exhausted opencast oil shale mines.

5. Conclusion

Afforestation of alkaline spoil on post-mining areas with fast-growing trees such as silver birch is the best means for restoration of these landscapes. Growing trees improve soil properties rapidly, and the rhizosphere effect has an important role in soil development and improvement. Complex interactions between soil rhizosphere, and fine-root morphology enhance the formation of sustainable and productive silver birch forests.

During the first 40 years of soil development, pH decreased one unit. Tree P nutrition improved already by the seventh year. During the first 30 years of stand development, soil N% and SIR increased tenfold; hence, soil fertility was remarkably improved. Rhizosphere effect indicating plant support to rhizosphere microbial communities was low a year after planting, highest in 2- and 3-year-old stands when soil conditions were still poor, but trees had already overcome the transplant shock, and decreased thereafter rapidly with age. All short-root morphological parameters showed certain trends with age. Short-root SRL, an indicator of rapid root growth and a key component of efficient capture of P, decreased logarithmically with stand age simultaneously with improving soil conditions. Diversity of bulk soil bacterial communities increased logarithmically with stand age. The rhizodeposition shaping the structure of the rhizosphere bacterial communities was massive because the similarities between rhizosphere and bulk soil bacterial communities soil were low, remaining in the range of 33–42% only. The results imply that silver birch has efficient root and rhizosphere adaptation strategies to cope with harsh conditions of abandoned opencast mines, and it is a promising tree species for reclamation of alkaline mining spoil.

Acknowledgments

This study was supported by Grants No. 7792 and 7452 of the Estonian Science Foundation, by Target Projects No. SF0180127508 and SF0182732506 of the Ministry of Education and Research of the Republic of Estonia, and by the EU through the European Regional Development Fund (Center of Excellence FIBIR). We thank Mr. Ilmar Part for linguistic revision of the manuscript.

References


Dilly, O., Bach, H.J., Buschor, F., Eschenbach, C., Kutsch, W.L., Middlehoff, U., Pritsch, K., Munch, J.C., 2000. Characteristics and energetic strategies of the rhizo-
Frouz, J., Novakova, A., 2005. Development of soil microbial properties in topsoil layer during spontaneous succession in heaths after brown coal mining in rela-
tion to humus microstructure development. Geoderma 129 (1–2), 54–64.
Insam, H., Domsch, K.H., 1988. Relationship between soil organic carbon and micro-
bial biomass on chronosequences of reclamation sites. Microbial Ecol. 15 (2), 177–188.
Oleskyn, J., Zytkowski, R., Reich, P.B., Tjoelker, M.G., Karoliewski, P., 2000. Ontoge-
Ostonen, I., Lõhmus, K., Lass, R., 1999. The role of soil conditions in fine root ecomor-
phological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. Tree Physiol. 27 (11), 1627–1634.
Trocha, L.K., Mucha, J., Eissenstat, D.M., Reich, P.B., Oleksyn, J., 2010. Ectomyc-
Withington, J.M., Reich, P.B., Oleksyn, J., Eissenstat, D.M., 2006. Comparisons of struc-