Black alder as a promising deciduous species for the reclaiming of oil shale mining areas

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Abstract

Black alder (*Alnus glutinosa*) plantations of different age were investigated on reclaimed oil shale mining areas in Northern Estonia with the aim of analysing the impact of microbial communities in the rhizosphere and bulk soil, and the effect of fine root adaptations on stand development and productivity. Biolog Ecoplates were used to determine the community-level physiological profiles (CLPP) in the rhizosphere and bulk soil samples. CLPP were summarized as AWCD (average well color development, OD $48h^{-1}$). The rhizosphere/bulk soil ratio of AWCD was an order higher in the 4-yr-old stand than in the first year after planting or in middle-aged stands. The substrate-induced respiration (SIR) and basal respiration (BAS) of bulk soil samples were measured, and the metabolic quotient $q(CO_2)$=BAS/SIR was calculated. Short root morphological studies were carried out using WinRHIZO™ Pro 2003b. SIR increased from 0.23 to 2.73 $mgCg^{-1}$, while $q(CO_2)$ and mean specific root length (SRl, m g^{-1}) decreased with increasing stand age (from 1 to 26 years) from 0.51 to 0.25, and from 172 to 90 m g^{-1} respectively. Soil pH decreased 1 unit during the first 26 years. According to efficient adaptive strategies, the survival and productivity of black alder stands on oil shale mining areas are high, and hence black alder is a perspective tree species for the afforestation of these areas.
Keywords: Alnus glutinosa, Biolog Ecoplates, fine root adaptations, oil shale mining area, rhizosphere processes, substrate-induced and basal respiration.

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1 Introduction

1.1 Reclamation of disturbed landscapes in Northern Estonian oil shale mining area

Every year opencast mining in the Northeast Estonian oil-shale field – the largest commercially exploited oil-shale deposit in the world (>6600 million tons) – creates substantial areas of wasteland. Presently, 2 underground mines and 2 open pit mines are in operation. In this area the overburden reaches ranges of 0-70 m due to the gentle southward dipping of the Ordovician strata. Mining in pits began to spread intensively in 1959. Pits are used in digging up to depth of 40 m; if oil shale is deeper than 50 m underground, mining is used. Nowadays around 50% of oil shale is mined in pits. The relief of the alkaline (pH~8) wasteland is rugged, the soil heterogeneous and extremely stony. Stone content varies from 15 to 100%; the N and organic content of oil shale mining spoil is low. Hence, afforestation is an optimal tool for the reclamation of these disturbed landscapes, as well as being a sustainable management option to create renewable energy sources.

The extensive afforestation of exhausted opencast oil-shale mines in Northeast Estonia was begun in 1960, and on the 1st of January 2005, exhausted oil-shale mines covered 12,900 ha of land, of which 10,200 ha had been forested. Until recent years a disproportionately large proportion of conifers (>90%), mainly Scots pine (*Pinus sylvestris*), has been planted (86% of the area). A total of 52 indigenous and introduced woody species were planted, where different introduced species of *Larix* (*Larix europaea*, *L. sibirica*, *L. kurilensis*) showed the best growth among coniferous trees, and the native deciduous species silver birch (*Betula pendula*) and black alder (*Alnus glutinosa*) were the most successful deciduous trees. They exceed pine stands of the same age (25-30 years) by 4 to 7 metres in height. The recommended planting density for black alder is 2000 - 2500, and hence thinning at a young age is unnecessary. In very stony areas (stones compromise 50-70%), black alder. is more suitable to promote soil formation processes and surpassed the growth of silver birch.

1.2 Advantages of alders among woody species

Alders have a number of advantages over other cultivated species: the increased N and P availability in the soil under alders, faster growth at a young age and higher resistance to pests, diseases and fires, especially in comparison with conifer monocultures. Owing to the ability to fix N_2 by the symbiosis of actinomycetes *Frankia* in alder root nodules, the soil under alders is enriched in nitrogen. Due to the low nitrogen retranslocation from senescing leaves (from 2.5 to 14%), alder leaf litter is extremely rich in nitrogen and mineralises easily [1, 2]. Alders increase phosphorus availability in the soil through the activity of their roots and associated microbial communities [3, 4]. Thus alders improve both soil N and P status. The majority of alder roots with primary structure are ectomycorrhizas, and hence mycorrhizal symbiosis plays an important role in the nutrient cycling of alder stands. The nutrient demand of alders is higher and N use efficiency is lower than that of other tree species. In order to satisfy the high demand for nutrients, beneficial rhizosphere conditions should be supported by alders [5].

1.3 Rhizosphere processes and fine-root adaptations in black alder

The potential of black alder for the recultivation of exhausted opencast oil shale mines in relation to fine-root adaptations and rhizosphere processes is still poorly understood. Populations of microbes in the rhizosphere differ quantitatively and qualitatively from those in the bulk soil; their numbers are generally higher, and different populations are commonly represented [6]. It has been shown that, in black alder, the size of the total microbial population and the numbers of ammonifying and proteolytic microorganisms are higher in the soil-root interface than in bulk soil, litter, and root-free soil under trees [7]. 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 of roots and soil microbial communities should be especially important in harsh site conditions, including reclaimed opencast oil-shale-mining areas [5].

Biolog Ecoplates, where all substrates are known as root exudates, were used to determine the community-level physiological profiles (CLPP) of culturable bacteria in soil-root interface and bulk soil samples [8]. The method characterizes the part of culturable bacteria and not fungi, but in studied alder sites the fungal/bacterial ratio [9] should be low, due to the soil pH_{KCl} range (from 7.0 to 8.0). An advantage of Biolog microplates is that they allow one to assess the functional diversity of the culturable microbial community. It is assumed that higher activity and diversity values for culturable microbial communities correspond to respectively higher values of total rhizosphere communities and can be used as indicators of their activity and diversity. In order to estimate the soil microbial biomass and metabolic activity of humus-degrading microorganisms present in the bulk soil, respiration techniques SIR and BAS were measured [10–12].

In considering microbial communities in the rhizosphere, the morphological and functional variability of fine roots is important. The impact of fine-root morphological parameters on rhizosphere processes in trees has not yet been thoroughly investigated. We measured short-root size and functional parameters: specific root area (SRA) and specific root length (SRL) [5, 13, 14] in alder stands. The results of different approaches: measurements of the size and activity of microbial communities by respiration in soil, the assessment of culturable bacterial communities with Biolog Ecoplates, the foliar assimilation efficiency of the above-ground part of trees, and short root morphology, especially the functional parameter SRA, were in close accordance [5].

The impact of alder species on fine-root morphology was significant, and short root tips were larger for black alder than or grey alder. For the investigated microbiological characteristics, no alder-species-related differences were revealed. The AWCD values of culturable bacterial communities in the soil-root interface compared to those in the bulk soil correlated positively with specific short-root area and negatively with foliar assimilation efficiency [5]. The first years of stand development are most critical for tree survival, and it is for that reason that one-, four- and 27-yr-old stands were included in the study. The higher growth rate of black alder corresponds to the higher activity of microbial communities in their rhizosphere, and the higher specific root area of short roots than in the case of conifers [5, 14, 15].

1.4 Aims and objectives of study

(1) To analyse the impact of microbial communities in the rhizosphere and in the bulk soil, and of fine-root adaptations on the development and productivity of black alder stands on reclaimed oil-shale mining areas.
(2) To make recommendations concerning the suitability of black alder for the recultivation of exhausted oil-shale opencast mines.

2 Materials and methods

2.1 Stand and soil characteristics

A one-yr-old black alder plantation (Narva II, 3 replication plots) established in 2005 and a 4-yr-old plantation (Narva I) established in 2002 on oil shale mining spoil were investigated in October 2005 and 2004 respectively. A middle-aged Sirgala stand was established in 1978 [15] the present soil type is *Spolic Anthrosol* [14], and the stand was involved in the study in October 2002 and 2004. The planting arrangement was in all cases 2x2 m, bare-root seedlings were one year old in Sirgala and Narva I, and in Narva II were two years old. Stand and soil characteristics are presented in Table 1, initial N and organic content is low, and in young stands a significant proportion of the organic matter is formed from oil shale mining residues [16].

Table 1: Stand and soil characteristics in 2005. DBH (cm) – diameter at breast height (*indicates diameter at root collar); H- tree height (m), N% and LOI% indicate the percentage of nitrogen and loss on ignition respectively.

Stand	Age	Trees per ha	DBH	H	N%	LOI%
Sirgala	27	1650	14.8	20.3	0.55	15.0
Narva I	5	2100	*2.8	0.98	0.04	1.3
Narva II	1	2300	*0.8	0.33	0.03	4.7

2.2 Soil sampling and processing

Ten samples from the 0-10 cm soil layer (20x20 cm^2) were taken randomly in the Sirgala black alder stand in October in 2002 and 2004. One third of the root system was taken from the 4-yr-old Narva II stand, and the whole root system with the soil of 10 randomly selected trees was taken from the one-year-old Narva I stand (from all 3 replicate plots) in October 2004 and 2005, respectively. A compound subsample per plot was processed according to the methodology proposed by Gobran and Clegg [17]. Gobran and Clegg [17] introduced a conceptual model for nutrient availability in the mineral soil-root system, in which the fine roots and associated organisms maintain a higher nutrient availability in the soil-root interface than in the bulk soil.

All roots were carefully removed by hand from the field-moist mineral soil, which was then passed through a 2-mm mesh sieve to yield the bulk soil fraction. The dead roots, nodules, and coarse roots (≥ 2 mm in diameter) were separated; the remaining fine roots (d < 2 mm) and soil were gently shaken for 1 min in a plastic container to separate the soil aggregates from the roots. The remaining fine roots with adhering soil yielded the rhizosphere fraction.

2.3 Chemical analysis

Nitrogen in the rhizosphere and bulk soil samples was determined using the Kjeldahl method with a Tecator ASN 3313. Loss on ignition (LOI) was determined at 360°C; the pH_{KCl} of samples was measured. Analyses were performed at the Biochemistry Laboratory of the Estonian University of Life Sciences.

2.4 Microbiological methods

Biolog Ecoplates (Biolog Inc.) where all carbon sources are known as root exudates were used to determine the community-level physiological profiles of culturable bacterial samples in the soil-root interface and bulk soil; 1g of fresh material was used in all cases, and the data were recalculated on the basis of the dry matter. Biolog profiles were summarized as AWCD (average well color development). AWCD - average well color development; this is the sum of all 31 substrates' utilization values by culturable bacteria, divided by 31. A 150-µl aliquot of a 10^{-4} dilution of the bulk soil or soil-root interface sample was added to each of the 96 wells (31 carbon sources and control in 3 replications) in the microplate. Plates were incubated at 25ºC and colour development was measured every 24 h for 120 h as absorbance at 590 nm, with optical density plate reader Multiscan 340 C. Based on the examination of the kinetic curves of the AWCD, 48h measurements were chosen for further data analysis.

Active microbial biomass was determined using substrate-induced respiration (SIR) [10–12]. Microbial respiration activity (BAS) was measured by trapping the evolved carbon dioxide in sodium hydroxide. The carbon availability index, also called metabolic quotient $q(CO_2)$, which relates the respiration rate without added substrate (BAS) to respiration after the addition of sufficient readily available substrate (SIR), was also calculated.

2.5 Morphological parameters of fine roots

The fine-root parameters of black alder were measured in 10 samples per year and plot, and 2-3 subsamples were processed per sample. Only short roots with a living cortex were considered: the diameter, length, volume and mass of root tips, as well as specific root area and specific length, were estimated. The methods are the same as in [13], except for the diameter, root length and root projection area measurements, which in the present work were carried out using WinRhizo 2003B (Regent Instruments) [14].

2.6 Statistical methods

Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilk's tests were used to check the normality of variables. When necessary, log- and root-transformations were used to normalize the data. Differences between stand means of short-root characteristics were checked at 95% confidence intervals. The level of significance of $\alpha = 0.05$ was accepted in all cases. The STATISTICA 7.0 software was used.

3 Results and discussion

3.1 Strategies to optimise mineral nutrition

To sustain and improve mineral nutrition in the harsh conditions of the levelled hills of oil shale mining spoil, trees must invest assimilates according to an extensive or intensive strategy. Concerning the optimality theory, the cost/benefit ratio of operating roots is a key factor for forest productivity [18].

There are two main strategies to optimise the mineral nutrition of plants:
A) Extensive, by increasing the mass, surface area and length of fine roots, leading to the increase of the rhizosphere.
B) Intensive, by increasing or maintaining the efficiency of fine roots and rhizosphere processes through morphological adaptations of fine roots [13, 19], and/or the activity of root-associated microorganisms for plant mineral nutrition could be increased or maintained.

Hence an extensive strategy leads to an increase in fine root system, but an intensive strategy is based on more efficient use of the mass unit of ectomycorrhizas.

3.2 Dynamics of fine root adaptations and pH in black alder stands

The mean specific root area of ectomycorrhizas was 171±12, 155±8, and 82±4 $m^2 kg^{-1}$ in one-, four- and 27-yr-old black alder stands respectively (Table 2), whereas in the 27-yr old stand the value was significantly higher.

The mean diameter and weight of a short root tip were significantly smaller in a one-yr-old plantation than in older stands, most probably as a result of planting shock. Mean specific root length decreased significantly in correlation with increasing stand age (Fig.1A).

Phosphorus nutrition was improved through the increasing of rhizosphere pH by up to 1.4 units compared to the bulk soil; soil pH decreased 1 unit during the first 26 years Fig. 1B).

Table 2: Mean short-root morphological parameters (± standard errors) in black alder stands. Different letters indicate significant differences between means by 95% confidence intervals, P<0.05.

Stand	Diameter (mm)	Length, (mm)	Weight, (mg)	SRA, (m^2 kg^{-1})
Sirgala 27 yr	0.291^a±0.005	3.02^a±0.18	0.035^b±0.003	82^a±4
Narva I 4 yr	0.392^c±0.005	4.10^b±0.19	0.034^b±0.002	155^b±8
Narva II 1 yr	0.321^b±0.008	3.51^a±0.38	0.021^a ± .002	171^b±12

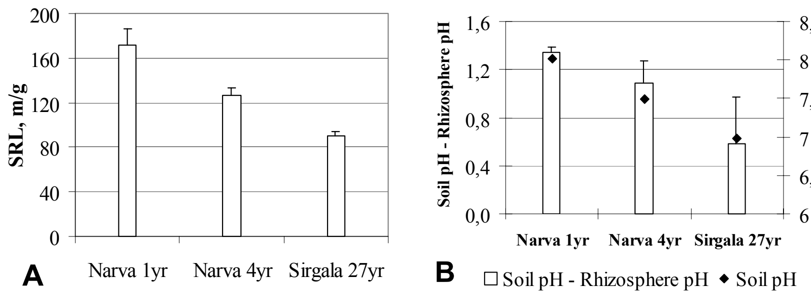

Figure 1: A: Decrease in mean SRL of short roots with increasing stand age; B: Acidifying effect of rhizosphere and soil pH(KCl) dynamics in black alder stands on reclaimed oil shale mining area. Bars indicate standard errors.

3.3 Activity dynamics of microbial communities in rhizosphere and soil

Oil shale mining spoil is extremely low in nitrogen and organic matter. Additionally, the microbial abundance is initially very low, as can be seen in Fig. 2A. The soil improvement during the first 25 years is remarkable (Table 1, Fig.1B and 2A).

The metabolic quotient $q(CO_2)$ was similar in two young stands, decreasing approximately twice during stand development (Fig. 2B) This reflects successional changes in microbial community structure, where in the soil of the young stands the microbial community consists mainly of r- strategist species that rapidly utilize root derived organic substrates and are not as efficient in the formation of biomass.

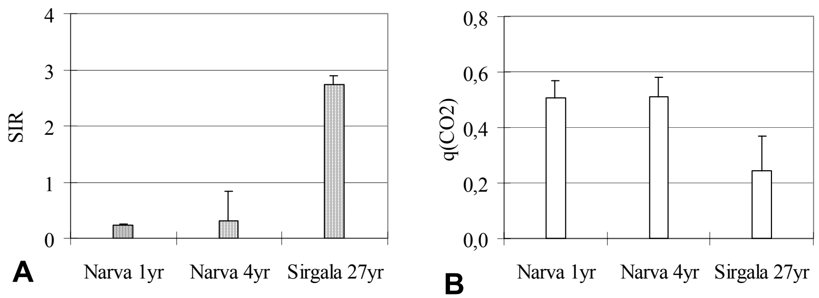

Figure 2: A: Dynamics of SIR (mg C g^{-1}); B: Decrease in metabolic quotient $q(CO_2)$ with increasing age of black alder stands on reclaimed oil shale mining area. Bars indicate standard errors.

In older stands where plant litter has been accumulating, the microbial community also contains K-strategic species that are characterized by a slow growth rate and stable biomass development.

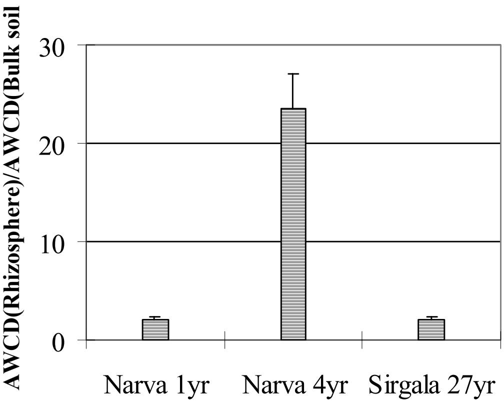

Figure 3: Rhizosphere /bulk soil AWCD ratio in black alder stands on reclaimed oil shale mining area. Bars indicate standard errors.

The greatest difference between one-, 4- and 27-yr-old stands was revealed in the rhizosphere/bulk soil AWCD ratio, which reflects the difference between activities of rhizosphere and bulk soil microbial communities (Fig. 3). Hence, in the second year after planting, when survived plants have overcome planting shock, the intensive strategy prevails, and plants support rhizosphere microbial communities massively. The rhizosphere/bulk soil activity ratio is an order higher than in the first year after planting or in a middle-aged stand. In our earlier investigation it was revealed that the better the soil conditions, the smaller the difference between the activities and diversities of microbial communities in the rhizosphere and soil. Hence, by improved soil the support of rhizosphere communities is less crucial.

Why are the rhizosphere communities poorly supported in the first year after planting, when the need is most urgent? It seems that during the first year the intensive strategy of the development of the fine root system prevails in order to exploit the oil shale mining spoil by fine roots as much as possible, and there is a deficit in assimilates allocated below ground. That priority is reflected by the highest short root SRL being found in the one-yr-old stand (Fig. 1A), although the SRA of ectomycorrhizas is similar in one-yr-old and 4-yr-old stands. That is in close accordance with the low support of rhizosphere microbial communities, because in our earlier investigation the Rhizosphere /bulk soil AWCD ratio, ratio for Shannon diversity indices, and SRA were positively correlated ($r=0.65$, $P<0.05$) in both cases [5].

In Sirgala, the analysis of topsoil nutrients showed a remarkable increase in total N and available P under the canopy of the black alder plantation in comparison to the neighboring Scots pine stand of the same age. A more than twice higher total topsoil N and available P content were found in the alder plantation compared to the pine stand [15]. The above-ground productivity in the 21-year-old black alder plantation on the reclaimed oil-shale mining area in Sirgala was comparable with the value for stands of the same age growing on fertile mineral soils. The biomass accumulation ratio, foliar assimilation efficiency and N use efficiency were highest in the Sirgala plantation. In Sirgala, the planting density ensured nearly optimal photosynthetic conditions, and the planting of 2,000 to 2,500 black alders per hectare on exhausted opencast oil-shale mines was recommended.

4 Conclusions

Black alder grows rapidly on recultivated oil shale areas; its survival after establishment is very good. A favorable environment for microbes in rhizosphere and bulk soil is created on oil shale mining detritus under black alders. The morphological adaptations of ectomycorrhizas support the effective functioning of the developing ecosystem. Therefore black alder is a very promising tree species for the recultivation of exhausted opencast oil shale mines.

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