Estonian Journal of Ecology, 2012, **61**, 4, 265–292 doi: 10.3176/eco.2012.4.03

Comparison of soil microorganism abundance and diversity in stands of European aspen (*Populus tremula* L.) and hybrid aspen (*Populus tremuloide*s Michx. × *P. tremula* L.)

Lelde Grantina-Ievina[a]✉, Dace Saulite[a], Martins Zeps[b], Vizma Nikolajeva[a], and Nils Rostoks[a]

[a] Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, 4 Kronvalda Blvd., Riga, LV-1586, Latvia
[b] Forest Tree Breeding Group, Latvian State Forest Research Institute 'Silava', 111 Rigas Str., Salaspils, LV-2169, Latvia

✉ Corresponding author, lelde.grantina-ievina@lu.lv

Received 22 June 2012, revised 1 October 2012, accepted 1 October 2012

Abstract. The use of short rotation forest tree species is increasing worldwide. The hybrid aspen (*Populus tremuloide*s Michx. × *P. tremula* L.) is one of the suitable tree species under the climatic conditions of the Baltic region. The cultivation of these trees on former agricultural soils differs from agricultural practices with reduced soil tillage and is characterized by increased demand of nutrients, which in long term can cause changes in the soil microbial populations. The aim of our investigation was to compare soil microbial populations in hybrid aspen and European aspen (*P. tremula* L.) stands in four sampling plots with aspen age ranging from 10 to 46 years. The abundance and diversity of soil microbial populations were estimated by enumeration of microorganisms (plate counts on three microbiological media) and by molecular methods (PCR, ARDRA, molecular identification of fungal isolates). Results showed that during long cultivation periods hybrid aspens reduced the number of culturable bacteria. The number of culturable filamentous fungi was statistically significantly increased only in one sampling plot in soil samples from hybrid aspen clones at a depth of 16–30 cm and only on one microbiological cultivation medium. The same was detected also with molecular methods in the case of fungal diversity estimated by Shannon–Weaver diversity indices in this sampling plot. None of the other characteristics of soil microbial populations, such as the number of yeasts and maltose utilizing bacteria on MEA, the number of yeasts and filamentous fungi on RBA, the total amount of soil DNA, fungal and bacterial diversity estimated by molecular biology methods, and species composition of filamentous fungi, was significantly affected by hybrid aspen. The identified filamentous fungi represented the following genera: *Acremonium*, *Exophiala*, *Geomyces*, *Gibellulopsis*, *Gibberella*, *Hypocrea*/*Trichoderma*, *Leptosphaeria*, *Metarhizium*, *Mortierella*, *Nectria*, *Paecilomyces*, *Penicillium*, *Trichosporon*, and others. The main conclusion was that cultivation of hybrid aspen as a short rotation forest tree in the Baltic region would not significantly affect the abundance and diversity of saprophytic soil microorganisms.

Key words: hybrid aspen, soil microorganisms, microbial diversity, soil filamentous fungi.

INTRODUCTION

The use of short rotation forest tree species has been increasing worldwide since the 1970s (Stanton et al., 2002). One of the suitable tree species for the climatic conditions of the Baltic region is hybrid aspen (*Populus tremuloide*s Michx. × *P. tremula* L.).

Hybrid aspen has been traditionally cultivated on former agricultural lands (since 1999 in Estonia) (Tullus et al., 2007) or on contaminated soils, and since 2000 in oil shale opencasts in Estonia (Tullus et al., 2008). Nevertheless, taking into account the possible environmental impacts of monospecific plantations with one tree species, cultivation of hybrid aspen is not recommended on traditional forest lands in Estonia (Tullus, 2010). In Latvia the genetic selection of hybrid aspen began in the 1960s and new scientific plantations have been established in recent decades (Zeps et al., 2008). At present there are over 4500 ha of hybrid aspen plantations in Northern Europe (Tullus et al., 2012).

Fast growing tree species can be favourable for the environment by decreasing soil erosion and surface runoff due to their root system and vegetation in comparison to conventional agriculture. This positive effect is detectable already in three to five years even in eroded soils (Mann & Tolbert, 2000). The possible adverse effects to the environment in the context of soil are connected with increased demand of nutrients. It is estimated that in poplar plantations the maximum uptake of nutrients occurs at the age of 5–6 years (Nelson et al., 1987), but for aspen stands in boreal climate it is observed that at the age of 9 years a rapid increase in annual biomass production occurs. Because of high establishment costs, hybrid aspen plantations are usually established with low planting densities. Thus the initial productivity per hectare is rather low and maximum nutrient uptake occurs at an older age compared to poplars and natural aspen stands; for example, the nutrient content in the above-ground biomass of 7-year-old hybrid aspen plantations could be only 0.5–3.5% of the total soil nutrient pool (Tullus et al., 2009). In addition, the growth of fast growing tree clones (*Salix* and *Populus* spp.) is characterized by increased litter amounts and an elevated C/N ratio and lignin content in the litter (Baum et al., 2009).

Cultivation of short rotation energy crops on former agricultural soils differs from agricultural practices with reduced soil tillage, which in long term can cause changes in the vertical distribution of soil microorganisms: an increased microbial biomass in the upper 5 cm of soil and a decreased biomass in subsoil (Makeschin, 1991). Cultivation of poplars and willows introduces ectomycorrhizal fungi in the former agricultural soils that normally contain saprophytic fungi and increases the diversity of basidiomycetes in general (Lynch & Thorn, 2006). In Germany Baum & Hrynkiewicz (2006) in their investigation of the rhizosphere and bulk soil of two willow clones (*Salix viminalis* and *S.* × *dasyclados*) differing in their mycorrhizal colonization and the decomposition rates of their litter found the species composition of saprophytic fungi to be tree clone specific. The lower mycorrhizal colonization and higher litter decomposition rate of *S. viminalis* brought about a higher number of saprophytic fungal species. The authors found

that colonization densities of seven saprophytic species (*Acremonium butyri*, *Cladosporium herbarum*, *Cylindrocarpon destructans*, *Penicillium janthinellum*, *Penicillium spinulosum*, *Plectosphaerella cucumerina*, and *Trichoderma polysporum*) significantly correlated with the acid-phosphatase and arylsulphatase activity in the rhizosphere. It is known that also *Populus* species and clones can differ in their mycorrhizal colonization rate and species composition as it was detected in the investigation of *Populus trichocarpa* and *P. tremula* L. × *P. tremuloide*s Michx. (Baum & Makeschin, 2000) and that hybrid aspen (*P. tremuloide*s Michx. × *P. tremula* L.) has an increased root biomass in comparison to European aspen (Nikula et al., 2009). Other investigations exist about the mycorrhizal colonization of *Populus* spp. (Cripps & Miller, 1995; Kaldorf et al., 2004) but there is a lack of information regarding the species composition of saprophytic fungi and bacteria in the soil of these trees. In the review article of Tullus et al. (2012) the authors conclude that there is a need for deeper investigations considering the environmental and biodiversity impact of hybrid aspen plantations.

The aim of our investigation was to compare soil microbial populations, mainly saprophytic fungi and bacteria, in hybrid aspen (*P. tremuloide*s Michx. × *P. tremula* L.) and European aspen (*P. tremula* L.) plantations on former agricultural lands. The hypothesis was that hybrid aspen plantations would cause changes in soil microbial composition especially in prolonged cultivation periods in comparison to European aspen plantations.

MATERIALS AND METHODS

Description of aspen and hybrid aspen stands and soil sampling

Soil in four hybrid aspen and four European aspen stands growing on former agricultural soils was analysed (Fig. 1). Information about the stands, tree age, and sampling time is given in Table 1. In each stand 10 soil samples (500 g) were

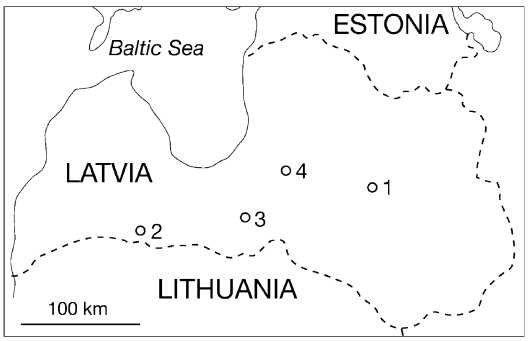

Fig. 1. Location of the studied stands. 1 – Forest Research Station 'Kalsnava', Kalsnava municipality; 2 – Forest Research Station 'Auce', Ukri municipality; 3 – private land, Iecava municipality; 4 – private land, Ropazi municipality.

Table 1. Characterization of the sampled aspen stands

Location	Year of planting and productivity		Characterization	Time of soil sampling
	Hybrid aspen	European aspen		
Forest Research Station 'Kalsnava', Kalsnava municipality	1964[a]	1966[a]	Hybrid aspen (1.1 ha) and European aspen (1.1 ha) in separate stands. Every five years offshoots are cut out	23.08.2010
Forest Research Station 'Auce', Ukri municipality	2000; 87 m^3 ha^{-1}[b]	2000; 39 m^3 ha^{-1}[b]	In parcels (5 × 5 trees), 1100 trees ha^{-1}	15.09.2010
Privately owned land, Iecava municipality	1998; 215 m^3 ha^{-1}[b]	1998; 60 m^3 ha^{-1}[b]	In parcels (3 × 5 trees), 2500 trees ha^{-1}	30.08.2011
Privately owned land, Ropazi municipality	2001[c]	2001[c]	In parcels (5 × 5 trees), 1100 trees ha^{-1}. European aspen clones were triploid	11.10.2011

[a] These data are not comparable due to the management activities that include tree crown forming.
[b] Average productivity estimated at the age of 12 years.
[c] Not estimated yet.

taken in the upper soil layer (0–15 cm) and 10 in the deeper soil layer (16–30 cm). In Kalsnava soil samples were taken diagonally through both stands. In other stands samples were taken randomly from various aspen clones planted in parcels in the middle of every parcel. All together 160 soil samples were analysed. Samples were placed in sterile plastic bags (*Nasco* WHIRL-PAK) and stored at +4 °C for a few days until the plate count of culturable microorganisms was made. After that the samples were stored at –20 °C.

Physical and chemical analyses of soil

Soil moisture content was determined according to the ISO 11465 standard method for every soil sample. The pH of the soil samples was measured in distilled water according to the method ISO 10390. Soil physical and chemical analyses of four soil samples from every aspen stand in three replicates were made in the laboratory of Forest Regeneration and Establishment Group of the Latvian State Forest Research Institute 'Silava'. The following methods were used: LVS ISO 11464

(2006) Soil quality – Pretreatment of samples for physico-chemical analysis, LVS ISO 11465 (2006) Soil quality – Determination of dry matter and water content on a mass basis – Gravimetric method, LVS ISO 11277 (2010) Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation, LVS ISO 11261 (2002) Soil quality – Determination of total nitrogen – Modified Kjeldahl method, LVS ISO 10694 (2006) Soil quality – Determination of organic and total carbon after dry combustion (elementary analysis), LVS ISO 10693:1995 Soil quality – Determination of carbonate content – Volumetric method, LVS ISO 11466:1995 Soil quality – Extraction of trace elements soluble in aqua regia, LVS 398 (2002) Soil quality – Determination of total phosphorus. The content of humic acids was determined according to the method of Zaccone et al. (2009).

Analysis of culturable soil microorganisms

In order to estimate the number of colony forming units (CFU) of culturable filamentous fungi (CFF), yeasts, and bacteria by the plate count method, soil sample dilutions were prepared by adding 10 g of soil to 90 mL of sterile distilled water. Suspensions were homogenized on a horizontal shaker for 1 h. After that serial dilutions were prepared, and 0.1 mL of dilutions 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were analysed. Agarised tryptic soy (Biolife, Italy) medium (TSA) was used for the enumeration of saprophytic bacterial CFU. The incubation time was 3 days, temperature 20 ± 2 °C. Agarised malt extract (MEA), 30 g/L, pH 5.5 (Biolife, Italy), was used as a growing medium that is favourable for fungi, yeasts, and maltose using bacteria. Rose Bengal agar (RBA) with chloramphenicol (Biolife, Italy) was used for the enumeration of fungi and yeasts. The incubation time for fungi was 5 days, temperature 20 ± 2 °C. The number of CFU was expressed per gram of dry soil.

Genera of CFF were determined after 10 days of incubation according to morphological characteristics and light microscopy results.

Extraction of total soil DNA and quality control

Total soil DNA was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., USA), which uses harsh lysis for the DNA extraction. Samples (250 mg) were homogenized using a horizontal Mixer Mill Type MM 301 (Retsch, Germany) at a maximal speed of 30 Hz (1800 oscillations/min) for 10 min. The amount and purity of the DNA were determined spectrophotometrically using Ultrospec 3100 Pro (Amersham Biosciences, UK) at wavelengths of 230, 260, and 280 nm in order to assess the contamination with proteins and humic compounds (Yeates et al., 1998).

ARDRA of soil DNA

For the amplified ribosomal DNA gene analysis (ARDRA) the fungal rRNA gene region that contains two internal transcribed spacers (ITS) and the 5.8S rRNA gene (ITS1-5.8S-ITS2) was amplified with the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990). In order to obtain the fingerprints of bacterial populations, the 16S region of bacterial ribosomal RNA was amplified with primers FORB and REVB (Edwards et al., 1989; Yeates et al., 1998).

The PCR reactions in Eppendorf Mastercycler Personal were carried out in 50 μL volume. The mixture contained 0.4 μL of Hot Start *Taq* DNA Polymerase, 5 μL of 10X Hot Start PCR Buffer, 5 μL of dNTP Mix, 2 mM each, 4 μL of 25 mM $MgCl_2$, 0.75 μL of Bovine Serum Albumin 20 mg/mL (all reagents from Fermentas, Lithuania), 1 μL of each 25 μM primer (OPERON Biotechnologies, Germany), 30.85 μL of sterile distilled water, and 1 μL of DNA template. The polymerase chain reaction (PCR) conditions were as follows: the initial denaturation step of 4 min at 95 °C, 40 s of denaturation at 95 °C, 40 s of annealing at 52 °C, 1 min of primer extension at 72 °C (30 cycles), and final extension 10 min at 72 °C.

For ARDRA analysis with restriction endonuclease *Bsu*RI (Chabrerie et al., 2003) the amplification products after the PCR were precipitated by 450 μL of 90% ethanol and 0.3 M sodium acetate (pH 5.0). The precipitated DNA was washed with 70% ethanol, air dried, dissolved in ddH_2O, and digested with *Bsu*RI (Fermentas, Lithuania). Restriction fragments were visualized in native 6% polyacrylamide gel electrophoresis using a Mighty Small^{TM} II (Hoefer, USA) unit. Gels were stained with ethidium bromide, photographed, and analysed with the software KODAK1D. For the calculation of the Shannon–Weaver diversity index (H') the following equation was used: $H' = -\sum p_j \log_2 p_j$, where p_j denotes relative intensity of an individual band (Gabor et al., 2003).

Sequencing of ribosomal DNA

A total of 221 isolates (88 sporulating and 133 white or black sterile mycelia, i.e. not sporulating when kept at +4 °C for several months) representing dominant filamentous fungi were isolated from the plates used for the enumeration of CFF and subcultured on MEA. Genomic DNA from approximately 0.25 g of mycelia was extracted using the method developed by Cenis (1992). The extracted DNA was amplified by PCR with primers ITS4 and ITS1F. The amplified DNA fragments from fungal isolates (101) from aspen stands in Kalsnava and Ukri were sequenced in CBS KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, in the frame of the project EMbaRC. After PCR 5 μL of each amplified product was subjected to a sequencing protocol with BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems, USA) with both primers at CBS and only with primer ITS1F in Latvia. The sequencing of the samples from Iecava and Ropazi was performed at the Latvian Biomedical Research and Study Center. Double stranded sequences of PCR amplicons were assembled using Staden Package 1.6.0. Homology search was done against the National Centre for

Biotechnology Information GenBank nucleotide database using the Basic Local Alignment Search Tool or against the Q-bank database (www.q-bank.eu).

Taxonomic data based on sequenced ribosomal DNA ITS regions of the isolates were used to calculate Sørensen's community similarity index C_s using the following equation: $C_s = 2C/A + B$, in which A and B are the number of species in samples A and B, respectively, and C is the number of species shared by the two samples (Izzo et al., 2006).

Statistical analyses

The *F*-test, *t*-test ($\alpha = 0.05$), and correlation analysis were made with *Excel* (Microsoft, USA). The microbiological data (number of CFU of fungi and bacteria, total soil DNA amount, as well as the results of ARDRA) were analysed for every geographical location separately; for example, the data from the hybrid aspen stand in Kalsnava were compared with the data from the European aspen in Kalsnava. The ten soil samples were treated as one data set ($n = 10$).

Multiple regression analysis was made with the R package (R Development Core Team, 2009). In the regression models stands of European aspen were labelled with 0 and stands of hybrid aspens with 1. Since soil chemical analyses were made only for four soil samples from each geographical location, average values of microbiological data of 10 samples from the same soil depth were used in multiple regression analysis ($n = 4$). Due to co-linearity of total nitrogen and total carbon values C/N ratios were used in the regression models.

RESULTS AND DISCUSSION

Physical and chemical characterization of soil

Data on the element content, total nitrogen content, and soil pH at both analysed depths of all soil profiles are listed in Table 2. Information on soil texture and moisture content is given in Table 3. The European aspen and hybrid aspen stands in Iecava are growing on soil that is chemically and granulometrically different from the soils of all other geographically distinct sampling plots. The upper soil layers down to 30 cm contained organic material, the soil there had the highest calcium content (39.30–71.32 g kg^{-1} versus 0.19–7.48 g kg^{-1} in other sampling plots) and the highest total carbon content (350.8–380.4 g kg^{-1} versus 10.8–39.0 g kg^{-1} in other places). In several cases the level of a particular element was reduced in the soil samples from hybrid aspen in comparison to the soil samples from European aspen (Table 2). For example, total nitrogen content and potassium content were reduced in all sampling plots except in Iecava. An increased C/N ratio in the soil of the hybrid aspen stand was observed only in Kalsnava, probably due to the age of this stand. This is in line with other investigations showing that hybrid aspen litter has an increased C/N ratio in comparison to European aspen litter (Nikula et al., 2009) followed by carbon sequestration in the soil (Baum et al., 2009).

Table 2. Element content, C/N ratio, the content of humic acids (n = 3), and soil pH at both analysed depths in all experimental plots. Asterisks indicate statistically significant differences between the soil samples from hybrid aspen (HA) and European aspen (EA) stands or parcels at the respective depths

Plot & depth, cm	Ca, g kg^{-1}	Mg, g kg^{-1}	K, g kg^{-1}	C_{total}, g kg^{-1}	P, g kg^{-1}	N_{total}, g kg^{-1}	C/N ratio	Humic acids, g kg^{-1}	pH_{H2O}
Kalsnava, HA									
0–15	7.48±0.505*	1.53±0.103	0.42±0.019*	21.4±1.6*	0.22±0.04	1.0±0.10*	21	13.15±0.01*	6.62±0.44
16–30	0.19±0.013*	0.95±0.064*	0.50±0.023*	19.5±0.7	0.19±0.03	0.8±0.00*	23	36.29±0.02*	6.03±0.16
Kalsnava, EA									
0–15	0.47±0.032*	1.69±0.114	0.82±0.037*	31.3±0.6*	0.17±0.03	1.7±0.00*	19	20.33±2.69*	6.70±0.21
16–30	0.63±0.043*	1.64±0.111*	0.68±0.031*	20.6±0.2	0.16±0.03	1.2±0.00*	17	24.57±2.06*	6.31±0.64
Ukri, HA									
0–15	0.59±0.040*	2.10±0.142*	2.49±0.114*	20.3±1.2*	0.12±0.02*	1.3±0.10*	15	50.66±0.75*	7.12±0.07
16–30	0.56±0.038*	2.31±0.156	4.02±0.183*	10.8±0.4*	0.12±0.02*	0.8±0.00*	13	75.72±9.64*	6.97±0.28
Ukri, EA									
0–15	1.49±0.101*	2.84±0.192*	1.78±0.081*	39.00±3.3*	0.18±0.03*	2.7±0.10*	14	43.14±2.01*	7.01±0.37
16–30	1.10±0.074*	2.21±0.149	2.10±0.096*	29.00±0.3*	0.17±0.02*	2.1±0.10*	14	47.81±1.84*	7.12±0.19
Iecava, HA									
0–15	70.43±4.757*	3.79±0.256*	0.52±0.024	380.2±3.90*	0.36±0.06	22.1±1.50	17	71.99±8.20	6.51±0.13
16–30	71.32±4.817	3.98±0.269	0.56±0.026	364.4±3.70*	0.35±0.06	23.3±0.40	16	73.88±23.26	6.69±0.09
Iecava, EA									
0–15	39.30±2.654*	3.26±0.220*	0.51±0.023	350.8±1.7*	0.35±0.06	22.1±1.60	16	73.86±12.75	6.65±0.24
16–30	68.10±4.599	3.75±0.253	0.56±0.026	370.2±1.2*	0.33±0.06	22.4±2.70	17	77.30±7.65	6.69±0.25
Ropazi, HA									
0–15	0.30±0.020*	0.35±0.024*	0.41±0.019*	18.3±0.6*	0.10±0.02*	1.4±0.10*	13	15.88±0.24	6.23±0.08
16–30	0.41±0.028*	0.36±0.024*	0.40±0.018*	15.1±0.4*	0.11±0.02*	1.0±0.00*	15	15.51±1.31*	6.08±0.23
Ropazi, EA									
0–15	0.79±0.053*	0.84±0.057*	0.87±0.040*	35.6±3.2*	0.23±0.05*	2.2±0.20*	16	26.36±11.78	6.75±0.40
16–30	0.38±0.026*	0.51±0.034*	0.46±0.021*	27.3±1.2*	0.19±0.04*	1.7±0.10*	16	20.08±0.34*	6.29±0.19

Table 3. Granulometric content ($n = 3$), soil texture class and soil moisture content ($n = 10$) at both analysed depths in all experimental plots. Asterisks indicate statistically significant differences between the soil samples from hybrid aspen (HA) and European aspen (EA) stands or parcels at the respective depths

Plot & depth, cm	Clay, %	Silt, %	Sand, %	Soil texture class	Soil moisture content, %
Kalsnava, HA					
0–15	3.1 ± 0.1*	19.3 ± 0.9*	77.6 ± 2.1*	Loamy sand	25.74 ± 12.07
16–30	4.9 ± 0.2*	23.6 ± 1.1*	71.4 ± 1.9*	Sandy loam	26.95 ± 12.65
Kalsnava, EA					
0–15	8.9 ± 0.4*	25.0 ± 1.1*	66.1 ± 1.8*	Sandy loam	21.72 ± 2.07
16–30	8.1 ± 0.3*	28.2 ± 1.3*	63.8 ± 1.7*	Sandy loam	17.30 ± 1.94
Ukri, HA					
0–15	17.2 ± 0.7	28.3 ± 1.3	54.4 ± 1.5	Sandy loam	15.61 ± 7.58
16–30	24.7 ± 1.0*	28.5 ± 1.3	46.7 ± 1.3*	Loam	12.27 ± 2.45
Ukri, EA					
0–15	17.5 ± 0.7	28.7 ± 1.3	53.8 ± 1.4	Sandy loam	24.96 ± 19.48
16–30	15.8 ± 0.6*	31.7 ± 1.4	52.5 ± 1.4*	Sandy loam	14.57 ± 2.97
Iecava, HA					
0–15	–[a]	–	–	–	50.06 ± 3.85
16–30	–	–	–	–	49.28 ± 7.02
Iecava, EA					
0–15	–	–	–	–	51.85 ± 3.86
16–30	–	–	–	–	52.75 ± 3.85
Ropazi, HA					
0–15	2.8 ± 0.1*	18.9 ± 0.9*	78.3 ± 2.1*	Loamy sand	22.49 ± 2.50
16–30	1.9 ± 0.1*	18.3 ± 0.8*	79.8 ± 2.2*	Loamy sand	16.32 ± 1.98
Ropazi, EA					
0–15	4.5 ± 0.2*	24.4 ± 1.1*	71.0 ± 1.9*	Sandy loam	25.37 ± 8.39
16–30	4.5 ± 0.2*	23.0 ± 1.0*	72.5 ± 2.0*	Sandy loam	16.66 ± 4.85

[a] The soil of these sampling plots was drained peat soil that contained only organic material at a depth of 0–30 cm.

Analysis of culturable microorganisms

The count of culturable microorganisms on three microbial media is given in Fig. 2a–e. The following statistically significant differences between the soils of hybrid aspen and European aspen stands were detected: a significantly higher number of bacterial CFU on TSA in the European aspen stand at Kalsnava ($F = 1.13$, $p = 0.02$; 2.14×10^6 CFU g^{-1} of dry soil versus 1.56×10^6 CFU g^{-1} of dry soil), significantly higher numbers of yeasts and maltose utilizing bacteria on MEA in the European aspen stand at Kalsnava ($F = 1.52$, $p = 0.003$; 4.86×10^6 CFU g^{-1} of dry soil versus 1.80×10^6 CFU g^{-1} of dry soil), and significantly higher numbers of filamentous fungi CFU on MEA at a depth of 16–30 cm in the soil of the hybrid aspen clones at Ropazi ($F = 12.27$, $p = 0.007$; 6.41×10^4 CFU g^{-1} of dry soil versus 3.23×10^4 CFU g^{-1} of dry soil).

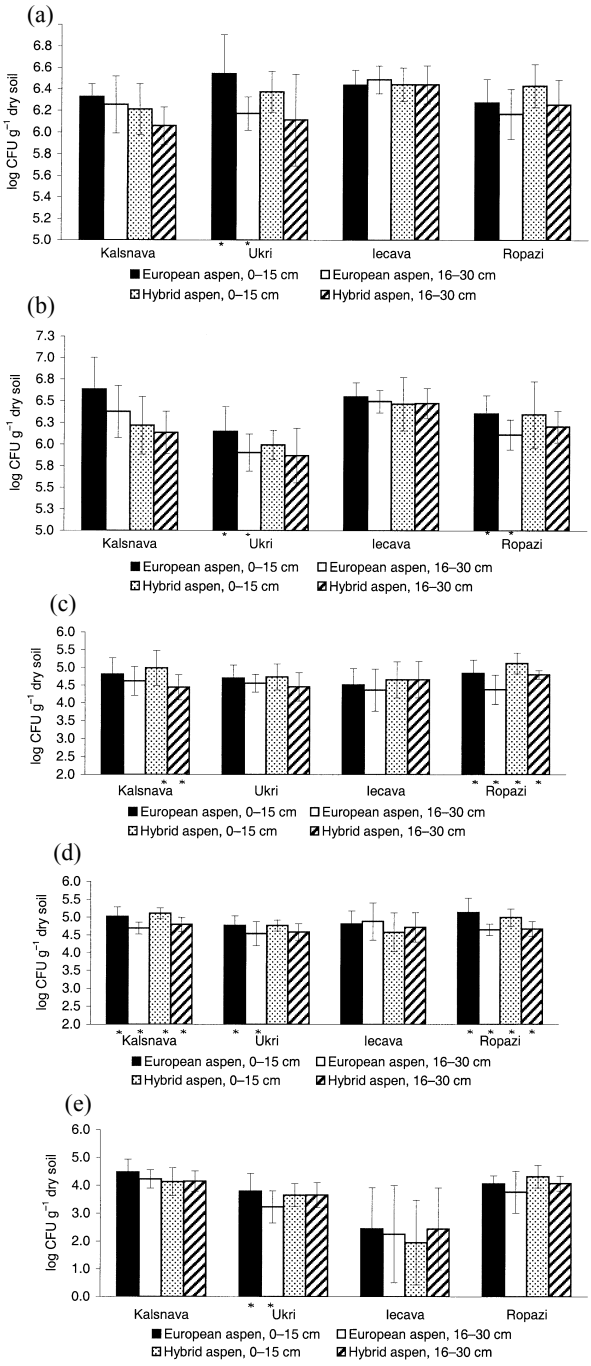

Fig. 2. Number of CFU of bacteria on TSA (a), yeasts and maltose utilizing bacteria on MEA (b), fungi on MEA (c), fungi on RBA (d), and yeasts on RBA (e) at both sampling depths in all aspen stands (± SD; $n = 10$). Results were obtained after three (in the case of bacteria) or five days (in the case of fungi) of incubation at 20 ± 2 °C. Asterisks indicate statistically significant differences ($p < 0.05$) between the sampling depths.

The total number of soil bacteria fluctuated from 5.37 to 6.99 log CFU g^{-1} of dry soil, which is similar to the total number of bacteria in agricultural soils, where it ranges from 5.10 to 7.85 log CFU g^{-1} of dry soil (Grantina et al., 2011a). The number of yeasts and maltose utilizing bacteria on MEA ranged from 5.34 to 6.71 log CFU g^{-1} of dry soil and the total number of CFF was from 3.93 to 5.92 log CFU g^{-1} of dry soil. These numbers are on average slightly higher than in agricultural soil where they fluctuate from 3.72 to 6.14 log CFU g^{-1} of dry soil and from 2.53 to 4.89 log CFU g^{-1} of dry soil, respectively (Grantina et al., 2011a), being more similar to forest soils where the level of CFF is 3.24–5.59 log CFU g^{-1} of dry soil in spruce forest (Grantina et al., 2012) and 3.58–5.08 log CFU g^{-1} of dry soil in mixed and pine forests (Grantina et al., 2011b).

In general the estimated CFU values represent the minimal number for CFF, because the method does not differentiate between colonies formed by single fungal spores and colonies formed by multicellular pieces of mycelium (Matthies et al., 1997). Moreover, as only a small number of bacteria (Torsvik et al., 1996) and fungi (Bridge & Spooner, 2001) are culturable, we used molecular biology methods to target also unculturable microbial populations.

Taxonomic analysis of soil fungal populations

According to the plate count method, the dominant genera of filamentous fungi were *Penicillium*, *Trichoderma*, *Paecilomyces*, *Mortierella*, and *Mucor* (Table 4). No statistically significant differences were observed between the soils of hybrid

Table 4. Average percentage of the representatives of five dominant fungal genera in the experimental plots (number of soil samples from each depth $n = 10$)

Experimental plot	Depth, cm	*Penicillium* spp., %		*Mortierella* spp., %		*Trichoderma* spp., %		*Paecilomyces* spp., %		*Mucor* spp., %	
		RBA	MEA	RBA	MEA	RBA	MEA	RBA	MEA	RBA	MEA
Kalsnava,	0–15	40.83	25.46	0.00	0.68	8.22	6.74	4.68	40.73	1.14	1.15
European aspen	16–30	32.47	40.34	5.41	8.42	1.71	13.49	11.85	0.00	0.33	19.88
Kalsnava, hybrid	0–15	37.78	19.15	0.87	9.31	0.65	0.71	0.00	38.14	1.18	0.68
aspen	16–30	24.25	13.56	0.15	3.39	0.16	0.00	0.00	0.00	2.25	3.39
Ukri, European	0–15	19.97	36.39	4.24	8.16	1.21	7.53	12.78	0.00	0.43	0.25
aspen	16–30	20.01	45.34	13.63	0.05	1.70	0.05	4.95	0.00	1.73	0.82
Ukri, hybrid	0–15	30.62	29.88	14.89	1.36	1.14	1.24	0.00	0.00	1.08	0.13
aspen	16–30	12.04	32.86	0.88	9.28	1.13	2.31	35.65	0.00	3.24	0.02
Iecava, European	0–15	24.76	14.37	5.87	18.30	0.87	0.25	29.37	15.70	1.01	1.04
aspen	16–30	16.05	32.59	10.95	10.01	0.17	2.36	3.04	8.84	1.03	1.58
Iecava, hybrid	0–15	22.42	14.52	4.32	0.62	1.21	11.53	16.93	13.38	0.97	2.30
aspen	16–30	25.44	20.73	2.57	6.79	2.40	1.87	1.03	2.66	1.11	1.53
Ropazi, European	0–15	29.45	37.14	5.86	0.98	2.03	5.92	15.42	11.24	0.78	1.23
aspen	16–30	32.73	13.04	1.86	10.48	4.05	1.03	17.60	0.85	9.36	2.16
Ropazi, hybrid	0–15	19.69	45.80	4.99	7.19	12.47	2.38	8.69	6.53	0.85	2.45
aspen	16–30	10.38	17.22	10.30	5.06	9.90	3.24	1.66	31.40	0.42	1.54

aspen and European aspen stands. Probably the reason was the fluctuation of results among 10 samples from the same plot and the same soil depth (Table 4). The members of the *Penicillium* genus ranged from 10.38% to 40.83% on RBA and from 13.04 to 45.80% on MEA (Table 4). *Mortierella* spp. constituted from zero to 14.89% on RBA and from 0.05% to 18.30% on MEA. Representatives of the *Trichoderma* genus ranged from 0.16% to 12.47% on RBA and from zero to 13.49% on MEA. The variation in the abundance of *Paecilomyces* spp. was from zero to 35.65% on RBA and from zero to 40.73% on MEA. The abundance of *Mucor* spp. varied from 0.33% to 9.36% on RBA and from 0.02% to 19.88% on MEA. Representatives of these five dominant genera constituted from 26.81% to 65.60% of all culturable fungi on RBA and from 20.34% to 82.13% on MEA. Less abundant genera were *Talaromyces*, *Umbelopsis*, *Metarhizium*, *Beauveria*, and *Acremonium*. Sterile white and black mycelia were observed as well.

The list of fungal genera determined according to morphological characters or sequencing results is given in Table 5. From 221 isolates sequenced 93.66% were Ascomycota (representing 58 genera), 4.98% Zygomycota (three genera), and only 1.36% Basidiomycota (one genus); all these together included 95 species. The largest part of the fungi were saprophytic: *Acremonium felinum*, *Acremonium strictum* (Watanabe, 2002), *Cladosporium cladosporioides*, *Mucor hiemalis*, *Paecilomyces carneus*, *Penicillium montanense* (Jayasinghe & Parkinson, 2008), *Penicillium* spp. (Baldrian et al., 2011), etc. Several identified species are entomopathogenic according to the literature: *Beauveria geodes*, *Tolypocladium geodes* (Santos et al., 2011), *Metarhizium anisopliae* (Leger et al., 1992), *Paecilomyces carneus*, *P. marquandii* (Sevim et al., 2010), *Isaria fumosorosea* (Ayala-Zermeño et al., 2011), *Lecanicillium kalimantanense* (Sukarno et al., 2009). *Bionectria ochroleuca* is associated with soil mites (Renker et al., 2005). Certain species are characterized as plant pathogens: *Gibberella pulicaris* (Desjardins & Gardner, 1991), *Leptosphaeria* sp. (Pedras, 2011), *Neonectria radicicola* (Halleen et al., 2004), or pathogens of soil nematodes: *Plectosphaerella cucumerina* (Atkins et al., 2003). Several species are associated with aspen leaves and roots: *Sagenomella diversispora* (Samson et al., 2011), *Lophiostoma* sp. (Albrectsen et al., 2010), or soil of aspen stands: *Trichoderma rossicum* (Friedl & Druzhinina, 2012). Fourteen species were detected in the investigation of microfungal communities of white spruce (*Picea glauca* (Moench) Voss) and trembling aspen (*P. tremuloides* Michx.) logs at different stages of decay in disturbed and undisturbed sites in boreal mixedwood forests of Alberta, Canada (Lumley et al., 2001) (last column in Table 5). Several species have been recorded to be associated with other tree species than aspens. For example, *Neonectria macrodidyma* was isolated as a root pathogen from the roots of nursery cultivated *Pinus sylvestris* L. (Menkis & Burokiene, 2012). Six species have been isolated in the rhizosphere of *Salix* spp.; these include *Humicola fuscoatra*, *Mortierella alpina*, *Volutella ciliata*, *Penicillium janthinellum*, and *Plectosphaerella cucumerina*. The abundance of the last two species correlates with the acid-phosphatase and arylsulphatase activity in the rhizosphere and is so related with the phosphorus and sulphur cycle in the soil

Table 5. List of fungal genera determined in the soil of hybrid aspen (HA) and European aspen (EA) stands according to morphological characters (indicated by x) or ribosomal DNA sequencing (indicated by total number of isolates and GenBank accession numbers of the homologue sequence)

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Ascomycota											
Acremonium felinum (Marchal) Kiyuna, An, Kigawa & Sugiy.		1	3	3					AB540562.1	1058	–
Acremonium strictum W. Gams		1	2	1					U57671.1	1063, 1064, 1120	+
Acremonium sp.		x							–	–	–
Alternaria sp.				1	1		1		GU934500.1		–
Aporospora terricola J.C. Krug & Jeng			1						AF049088.1	–	–
Arthrinium sacchari (Speg.) M.B. Ellis								1	EU579803.1	–	+
Aureobasidium sp.		1							GQ906942.1	1116	–
Auxarthron umbrinum (Boud.) G.F. Orr & Plunkett				1					FR718876.1	–	–
Beauveria geodes (W. Gams) Arx				x	x	x	1		U19037.1	1187	–
Beauveria sp.				x	x	x	x	x	–	–	–
Bionectria levigata Schroers					1				AF210680.1	1202	–
Bionectria ochroleuca (Schwein.) Schroers & Samuels					2		1	1	GU934503.1	1190, 1196, 1247	–
Botryotinia fuckeliana (de Bary) Whetzel	1								HM989942.1	–	–
Cadophora finlandica (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew							2		EF093179.1	1248, 1259	–
Cladosporium cladosporioides (Fresen.) G.A. de Vries								1	HQ832794.1	–	+
Clonostachys divergens Schroers								1	GU934587.1	1268	–

Continued overleaf

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Dokmaia sp.			2	1					HQ631068.1	1134	–
Emericella bicolor M. Chr. & States/*Aspergillus bicolor* M. Chr. & States					1				EF652511.1	1194	–
Emericella foeniculicola Udagawa						1			AB249011.1	1192	–
Eremomyces langeronii (Arx) Malloch & Sigler					1				AB128973.1	1199	–
Eucasphaeria capensis Crous				1			1	2	GU934520.1	1182, 1184, 1232	–
Exophiala salmonis J.W. Carmich.		1	1	1			1		GU586858.1	1114, 1117, 1124, 1260	+
Fusarium merismoides var. merismoides Corda					1				EU860057.1	1204	–
Fusarium oxysporum Schltdl.			1						GU445377.1	1105	–
Fusarium sp.		x				1			HQ731631.1	1199	–
Geomyces pannorum (Link) Sigler & J.W. Carmich.	1								DQ189229.1	1130	+
Geomyces destructans Blehert & Gargas			1	1					GU999986.1	1113	–
Geomyces sp.				1					DQ402527.1	1088	–
Gibberella pulicaris (Fr.) Sacc.				1					FJ481029.1	1143	–
Gibellulopsis nigrescens (Pethybr.) Zare, W. Gams & Summerb.					3	1	1	5	HQ115693.1	1189, 1191, 1205, 1250, 1266, 1267, 1269	–

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Gliomastix murorum (Corda) S. Hughes					1				AB540556.1	1235	–
Humicola fuscoatra Traaen						1			AB625589.1	–	+
Humicola sp.		x							–	–	–
Hyalodendriella betulae Crous								1	EU040232.1	1243	–
Hypocrea koningii Lieckf., Samuels & W. Gams/*Trichoderma koningii* Oudem.		2							AJ301990.1	1131	+
Hypocrea lixii Pat.							2		AY605716.1, HQ259311.1	1230, 1245	–
Hypocrea pachybasioides Yoshim. Doi	1								GU934589.1	1127	–
Hypocrea viridescens Jaklitsch & Samuels/*Trichoderma viridescens* (A.S. Horne & H.S. Will.) Jaklitsch & Samuels		2							GU566274.1	–	–
Ilyonectria europaea A. Cabral, Rego & Crous							1	1	JF735294.1	1241	–
Ilyonectria rufa A. Cabral & Crous								1	JF735278.1	–	–
Isaria fumosorosea Wize								1	JF792885.1	1239	–
Kernia pachypleura Malloch & Cain								1	DQ318208.1	1186	–
Lecanicillium kalimantanense Kurihara & Sukarno								1	AB360356.1	1270	–
Lecanicillium sp.								1	AB378528.1	1242	–
Leptosphaeria conferta Niessl ex Sacc.								1	AF439459.1	1264	–
Leptosphaeria maculans (Desm.) Ces. & De Not.						1			M96663.1	1196	–
Leptosphaeria sp.		2	2			2			AY336132.1	1062, 1128	–

Continued overleaf

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Leptospora rubella				1					AF383951.1	–	–
Leucostoma persoonii (Nitschke) Höhn.					1				HM061319.1	1193	–
Lophiostoma sp.					1				HM116744.1	–	–
Metarhizium anisopliae (Metschn.) Sorokīn	x	1 x		1 x		1 x		x	HM055436.1	1112	–
Nectria lugdunensis J. Webster		1							DQ247778.1	1101	–
Nectria mariannaeae Samuels & Seifert			1						EU273515.1	1048	–
Nectria vilior Starbäck					1				U57673.1	1198	–
Neonectria macrodidyma Halleen, Schroers & Crous							1	1	HM036602.1	–	–
Neonectria radicicola (Gerlach & L. Nilsson) Mantiri & Samuels			1						AJ875336.1	1123	–
Neonectria ramulariae Wollenw.							2		JF735314.1	1262, 1263	–
Paecilomyces carneus (Duché & R. Heim) A.H.S. Br. & G. Sm.	1	2				1			AB258369.1	1140, 1126, 1188	+
Paecilomyces marquandii (Massee) S. Hughes			3	2	1	2	1	2	AB099511.1, AB114223.1, FR799470.1	1061, 1121, 1141, 1151, 1236, 1237, 1255, 1257	–
Paecilomyces sp.	x	x	x	x	x	x	x	x	–	–	–
Paraconiothyrium sporulosum (W. Gams & Domsch) Verkley			1						GU566257.1	1059	–

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Penicillium brasilianum Bat.								1	HM469396.1	1229	–
Penicillium canescens Sopp	2	1	7	1	1		7	7	FJ439586.1, FJ791141.1, AY373901.1, JF311911.1	1091, 1093, 1095, 1104, 1107, 1109, 1110, 1119, 1208–1216, 1223–1227	–
Penicillium corylophilum Dierckx	1								GU566277.1	1135	–
Penicillium janthinellum Biourge		1							AB293968.1	1097	–
Penicillium montanense M. Chr. & Backus								1	HQ157959.1	1228	–
Penicillium roseopurpureum Dierckx					1	2			JF311930.1, JN246027.1	1218–1220	–
Penicillium swiecicikii K.M. Zalessky						1		3	AJ608946.1	1217, 1222, 1253	–
Penicillium sp.	1						2		GU446648.1	–	–
Penicillium sp.	x	x	x	x	x	x	x	x	–	–	–
Plectosphaerella cucumerina (Lindf.) W. Gams			2						AJ492873.1	1103	–
Pochonia suchlasporia (W. Gams & Dackman) Zare & W. Gams					1			2	AB214658.1	1192, 1240	–

Continued overleaf

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Podospora appendiculata (Auersw. ex Niessl) Niessl								1	AY999126.1	1201	–
Pseudallescheria fimeti (Arx, Mukerji & N. Singh) McGinnis, A.A. Padhye & Ajello							1		AY879799.1	–	–
Pseudeurotium bakeri C. Booth				1					GU934582.1	1133	–
Pyrenochaeta acicola (Moug. & Lév.) Sacc.								1	*	1251	–
Pyrenochaeta inflorescentiae Crous, Marinc. & M.J. Wingf.			1				1		GU586851.1	1149	–
Rhizopycnis vagum D.F. Farr	1								HQ610506.1	–	–
Sagenomella diversispora (J.F.H. Beyma) W. Gams		1							GQ169318.1	1111	–
Scedosporium apiospermum Sacc. ex Castell. & Chalm.			1						AB567756.1	1051	–
Scytalidium lignicola Pesante								1	FJ914697.1	–	+
Talaromyces ucrainicus Udagawa						x	x	1 x	AY533695.1	1231	–
Tetracladium setigerum (Grove) Ingold							1		FJ000374.1	1265	–
Trichocladium asperum Harz	1		1						HQ115689.1, AY706336.1	1108	–
Trichocladium opacum (Corda) S. Hughes				1					AM292049.1	958	–
Trichoderma hamatum (Bonord.) Bainier							3		JN542526.1	1233, 1234	+
Trichoderma rossicum Bissett, C.P. Kubicek & Szakács							1	1	DQ083024.1, EU280089.1	1183, 1246	–
Trichoderma tomentosum Bissett				1					AY605737.1	–	–
Trichoderma sp.		x	x	x	x	x	x	x	–	–	–
Verticillium dahliae Kleb.					1			1	HQ839784.1	1197	–

Table 5. *Continued*

Species	Kalsnava		Ukri		Iecava		Ropazi		Homologue sequence, NCBI acc. No.	No. in MSCL[a]	Detected in Lumley et al., 2001
	HA	EA	HA	EA	HA	EA	HA	EA			
Verticillium sp.		x							–	–	–
Volutella ciliata (Alb. & Schwein.) Fr.			1			1			GU586855.1, HQ897802.1	1203	+
Wardomyces inflatus (Marchal) Hennebert								1	FJ946485.1	1252	+
Zalerion varium Anastasiou	1								AJ608987.1	1118	–
Zygomycota											
Mortierella alpina Peyronel	1	1						2	AJ271629.1, FJ478130.1, JN943023.1	1098, 1254, 1271	+
Mortierella clonocystis W. Gams						1			HQ630318.1	1200	–
Mortierella globulifera O. Rostr.							1		JN943800.1	1244	–
Mortierella sp.		3	1						FJ810149.1, FJ810151.1, DQ093725.1	1052	–
Mortierella sp.	x	x	x	x	x	x	x	x	–	–	–
Mucor hiemalis Wehmer							1		EU484263.1	1181	+
Mucor sp.	x	x	x	x	x	x	x	x	–	–	–
Umbelopsis sp.	x		x		x			x	–	–	–
Basidiomycota											
Trichosporon porosum (Stautz) Middelhoven, Scorzetti & Fell								1	AJ608971.1	–	–
Trichosporon sp.		2							FJ439589.1	–	–

[a] Microbial Strain Collection of Latvia.
* According to the Q-bank database.

(Baum & Hrynkiewicz, 2006). Several species have been isolated from the soil of boreal mixedwood forests in Canada (*P. janthinellum*, *Trichoderma hamatum*, *T. koningii*, *Geomyces pannorum*, *M. hiemalis*, *P. carneus* (De Bellis et al., 2007)) and/or in the soil of birch stands in central Finland (*M. hiemalis*, *Paecilomyces carneus*, and *Penicillium montanense* (McLean & Huhta, 2002)).

The most common fungal species in the list of sequenced isolates was *Penicillium canescens* with a total of 26 isolates (11.76% of all sequenced isolates). This particular species has not been detected in the other investigations of forest soil microflora mentioned in the previous paragraph. Together with other *Penicillium* species (40 isolates in total) this genus constituted 18.10% of the sequenced isolates. These results are in line with the results of the plate count, where the most abundant genus was *Penicillium*. Other common genera were *Paecilomyces* (15 isolates) and *Hypocrea*/*Trichoderma* (13 isolates) in all sampling plots, *Acremonium* (11 isolates, all from the sampling plots in Kalsnava and Ukri), *Mortierella* (10 isolates, in all sampling plots), *Gibellulopsis* (10 isolates, isolated as black sterile mycelia only in the sampling plots in Iecava and Ropazi), and *Leptosphaeria* (8 isolates, isolated as grey sterile mycelia in Kalsnava, Ukri, and Iecava).

Sørensen's community similarity indices C_s between each plot of hybrid aspen and European aspen are given in Table 6. The highest Sørensen's community similarity index between soil samples of the hybrid aspen stand and the European aspen stand was at Ukri – 0.39, in other sampling plots it ranged from 0.21 to 0.26. Indices C_s among all sampling plots were lower: from 0.07 (nearly complete dissimilarity between Ropazi and Kalsnava) to 0.22 (between Ukri and Kalsnava) (Table 7). The value of C_s between the fungal populations of all hybrid aspen

Table 6. Total number of fungal species, number of shared species, and values of Sørensen's community similarity indices C_s between each plot of hybrid aspen (HA) and European aspen (EA)

Sampling plot (total number of species)	Kalsnava HA (12)	Ukri HA (19)	Iecava HA (14)	Ropazi HA (23)
Kalsnava EA (15)	C_s = 0.22 (3 shared species)			
Ukri EA (17)		C_s = 0.39 (7 shared species)		
Iecava EA (15)			C_s = 0.21 (3 shared species)	
Ropazi EA (31)				C_s = 0.26 (7 shared species)

Table 7. Total number of fungal species, number of shared species, and values of Sørensen's community similarity indices C_s among all sampling plots

Sampling plot (total number of species)	Ukri (36)	Iecava (29)	Ropazi (54)
Kalsnava (27)	C_s = 0.22 (7 shared species)	C_s = 0.14 (4 shared species)	C_s = 0.07 (3 shared species)
Ukri (36)		C_s = 0.18 (6 shared species)	C_s = 0.11 (5 shared species)
Iecava (29)			C_s = 0.19 (8 shared species)

stands and all European aspen stands is 0.29 (21 shared species from 68 species of hybrid aspen stands and 78 species from European aspen stands).

All calculated Sørensen's indices revealed that the fungal populations in the analysed sampling plots were quite different from one another. This is in line with an investigation in Lithuania about fungal populations in the soil of seven polluted sites, where the highest value of Sørensen's indices was 0.37 although the total number of identified species was 158 (Pečiulytė & Dirginčiutė-Volodkienė, 2010).

Microbial diversity in soil based on ARDRA analysis

The amount of soil total DNA varied remarkably among sampling plots. The lowest amount of extracted DNA was in the sampling plots at Kalsnava and the highest amount was in the soil samples from Iecava (Fig. 3). Diversity indices calculated from the ARDRA data are given in Fig. 4. There were no statistically significant differences in the fungal and bacterial diversity between the two soil depths. For further statistical analyses the data from both soil depths were combined, but there were no significant differences in microbial diversity between the soil in the stands of hybrid aspen and the soil in the stands of European aspen. The only difference that approached the statistical significance was observed in the case of Ropazi: the average fungal diversity index H' was 2.33 in the hybrid aspen stand versus 2.01 in the European aspen stand (F = 1.27; p = 0.08). In this sampling plot statistically significant differences between the number of fungal CFU on MEA were observed at a depth of 10–30 cm comparing the soils from the hybrid aspen and European aspen stands. It was further corroborated by statistically different fungal diversity H' values of this soil level: – 2.55 versus 2.02 (F = 1.33; p = 0.03). In the case of fungal diversity versus bacterial diversity the difference among all four sampling plots was much greater than in the case of CFU numbers: significantly lower fungal diversity indices were obtained from the samples of Ukri in comparison with the samples of Iecava.

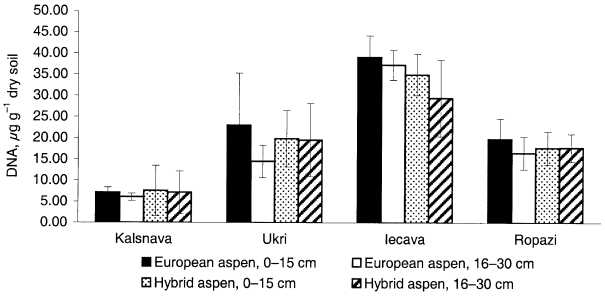

Fig. 3. Amount of total soil DNA in all sampling plots and at both analysed depths (± SD; $n = 10$).

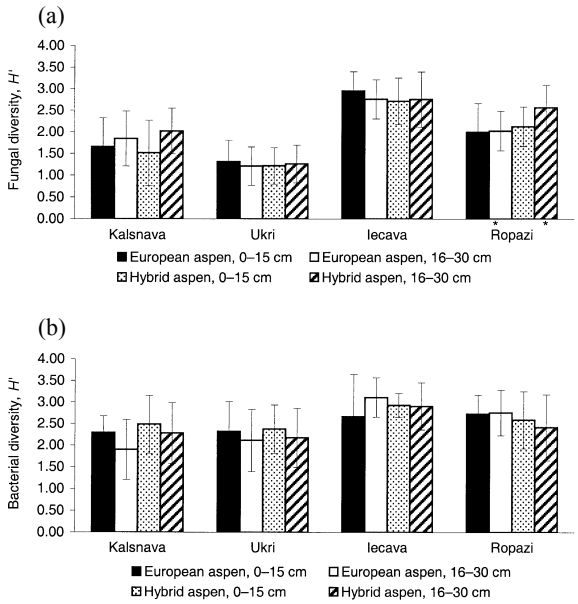

Fig. 4. Fungal (a) and bacterial (b) diversity indices H' in all sampling plots and at both analysed depths (± SD; $n = 10$). Asterisks indicate statistically significant differences ($p < 0.05$) between the sampling depths.

Multiple regression analyses of the impact of soil chemical characteristics on the microbial abundance and diversity

Considering that according to the *t*-test there were only a few statistically significant differences between the soil microbial populations of hybrid aspen and European aspen stands, soil chemical variables were included in multiple regression models in order to determine what parameters in general have a significant impact on the analysed soil microorganisms and on the amount of total soil DNA. The results of multiple regression analyses are summarized in Table 8. As it was

Table 8. Results of multiple regression analyses (*p*-values)

Factor	Total number of bacteria on TSA	Yeasts and maltose utilizing bacteria on MEA	Filamentous fungi on MEA	Yeasts on RBA	Filamentous fungi on RBA	Total amount of soil DNA	Fungal diversity, *H′*	Bacterial diversity, *H′*
Soil sampling depth	<0.0001[a]	NS	NS	NS	NS	NS	NS	NS
Aspen type	NS	NS	0.0005	NS	NS	NS	NS	NS
Soil moisture content	NS	<0.0001	0.0007	<0.0001	0.0005	<0.0001	<0.0001	NS
Soil pH	NS	NS	0.002	NS	0.02	NS	<0.0001[a]	0.02
Calcium content	NS	NS	NS	NS	NS	NS	NS	<0.0001
Magnesium content	0.0006	NS	NS	NS	0.003[a]	NS	NS	0.001[a]
Potassium content	0.0004[a]	NS	NS	NS	0.01	NS	NS	NS
Phosphorus content	0.02[a]	NS	NS	NS	0.046[a]	NS	NS	NS
C/N ratio	0.002[a]	NS	0.04[a]	NS	0.002	0.002[a]	0.02[a]	NS
Humic acids	NS	0.004[a]	<0.0001[a]	<0.004[a]	0.006[a]	0.03[a]	NS	NS
Multiple R^2	0.90	0.74	0.87	0.74	0.94	0.92	0.93	0.84
p-Value of the model	0.0001	0.0002	0.0003	0.0002	0.001	<0.0001	<0.0001	<0.0001

NS – not significant; [a] – negative impact.

detected with the *t*-test, the aspen type significantly affected the number of filamentous fungi on MEA but not the total number of bacteria on TSA. Although the soil pH values were similar in all sampling plots, the regression models showed that soil pH significantly influenced the number of filamentous fungi on MEA and RBA, and fungal and bacterial diversity indices H'. Of all the soil variables the most important were the C/N ratio and humic acid content. The C/N ratio values ranged from 13 to 23, and higher values of this ratio correlated with lower values of several microbiological variables such as the total number of bacteria on TSA, the number of filamentous fungi on MEA, the total amount of soil DNA, and fungal diversity. The concentration of humic acids in the soil negatively influenced the number of yeasts, maltose utilizing bacteria, and filamentous fungi on MEA, the number of yeasts and fungi on RBA, and the total amount of soil DNA. A similar regularity of the occurrence of a smaller number of yeasts in the soil layers with more humus was observed in the investigation of Priha et al. (2001) of soil microbial populations in pine, spruce, and birch stands in Finland.

CONCLUSIONS

1. In general, only a minor effect of hybrid aspen on soil microbial communities was observed. Our results show that during long cultivation periods (more than 40 years as in the case of the sampling plot at Kalsnava) hybrid aspens reduce the number of culturable bacteria, probably due to the increased C/N ratio in the soil. The number of culturable filamentous fungi was significantly increased only in one sampling plot (Ropazi) of hybrid aspen in the soil sampled at a depth of 16–30 cm and only on one microbiological cultivation medium. The same trend in the fungal diversity was detected in the same sampling plot also with molecular methods estimated by Shannon–Weaver diversity indices.
2. Hybrid aspen stands did not affect any other analysed characteristics of the soil microbial population, such as the number of yeasts and maltose utilizing bacteria on MEA, the number of yeasts and filamentous fungi on RBA, the total amount of soil DNA, the fungal and bacterial diversity estimated by the methods of molecular genetics, and species composition of filamentous fungi. So it is safe to cultivate hybrid aspen (*Populus tremuloide*s Michx. × *P. tremula* L.) as a short rotation forest tree species in the Baltic region if the abundance and diversity of saprophytic soil microorganisms is considered.

ACKNOWLEDGEMENTS

This study was supported by the project ‘Capacity building for interdisciplinary biosafety research’ No. 2009/0224/1DP/1.1.1.2.0/09/ APIA/VIAA/055 co-funded by the European Social Fund.

We are very thankful to the colleagues at CBS KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, and the project EMbaRC for the sequencing of fungal isolates from Kalsnava and Ukri.

REFERENCES

Albrectsen, B. R., Björkén, L., Varad, A., Hagner, Å., Wedin, M., Karlsson, J. & Jansson, S. 2010. Endophytic fungi in European aspen (*Populus tremula*) leaves – diversity, detection, and a suggested correlation with herbivory resistance. *Fungal Diversity*, **41**, 17–28.

Atkins, S. D., Clark, I. M., Sosnowska, D., Hirsch, P. R. & Kerry, B. R. 2003. Detection and quantification of *Plectosphaerella cucumerina*, a potential biological control agent of potato cyst nematodes, by using conventional PCR, real-time PCR, selective media, and baiting. *Appl. Environ. Microb.*, **69**, 4788–4793.

Ayala-Zermeño, M. A., Reyes-Montes, M. R., Arroyo-Vázquez, E., Calderón-Ezquerro, M. C., Mier, T., Robledo-Retana, T. & Toriello, C. 2011. An *Isaria fumosorosea* SCAR marker for evaluation of soil, insect, and airborne samples. *Biocontrol. Sci. Techn.*, **21**, 1091–1102.

Baldrian, P., Voříšková, J., Dobiášová, P., Merhautová, V., Lisá, L. & Valášková, V. 2011. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. *Plant Soil*, **338**, 111–125.

Baum, C. & Hrynkiewicz, K. 2006. Clonal and seasonal shifts in communities of saprotrophic microfungi and soil enzyme activities in the mycorrhizosphere of *Salix* spp. *J. Plant Nutr. Soil. Sc.*, **169**, 481–487.

Baum, C. & Makeschin, F. 2000. Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* × *tremuloides*). *J. Plant Nutr. Soil. Sc.*, **163**, 491–497.

Baum, C., Leinweber, P., Weih, M., Lamersdorf, N. & Dimitriou, I. 2009. Effects of short rotation coppice with willows and poplar on soil ecology. *Landbauforschung – vTI Agriculture and Forestry Research 3*, **59**, 183–196.

Bridge, P. & Spooner, B. 2001. Soil fungi: diversity and detection. *Plant Soil*, **232**, 147–154.

Cenis, J. L. 1992. Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Res.*, **20**, 2380.

Chabrerie, O., Laval, K., Puget, P., Desaire, S. & Alard, D. 2003. Relationship between plant and soil microbial communities along a successional gradient in a chalk grassland in north-western France. *Appl. Soil Ecol.*, **24**, 43–56.

Cripps, C. L. & Miller, O. K. 1995. Ectomycorrhizae formed *in vitro* by quaking aspen: including *Inocybe lacera* and *Amanita pantherina*. *Mycorrhiza*, **5**, 357–370.

De Bellis, T., Kernaghan, G. & Widden, P. 2007. Plant community influences on soil microfungal assemblages in boreal mixed-wood forests. *Mycologia*, **99**, 356–367.

Desjardins, A. E. & Gardner, H. W. 1991. Virulence of *Gibberella pulicaris* on potato tubers and its relationship to a gene for rishitin metabolism. *Genetics*, **81**, 429–435.

Edwards, U., Rogall, T., Blocker, H., Emde, M. & Bottger, E. C. 1989. Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res.*, **17**, 7843–7853.

Friedl, M. A. & Druzhinina, I. S. 2012. Taxon-specific metagenomics of *Trichoderma* reveals a narrow community of opportunistic species that regulate each other's development. *Microbiology*, **158**, 69–83.

Gabor, E. M., de Vries, E. J. & Janssen, D. B. 2003. Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. *FEMS Microbiol. Ecol.*, **44**, 153–163.

Gardes, M. & Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Mol. Ecol.*, **2**, 113–118.

Grantina, L., Kenigsvalde, K., Eze, D., Petrina, Z., Skrabule, I., Rostoks, N. & Nikolajeva, V. 2011a. Impact of six-year-long organic cropping on soil microorganisms and crop disease suppressiveness. *Žemdirbystė = Agriculture*, **98**, 399–408.

Grantina, L., Seile, E., Kenigsvalde, K., Kasparinskis, R., Tabors, G., Nikolajeva, V., Jungerius, P. & Muiznieks, I. 2011b. The influence of the land use on abundance and diversity of soil fungi: comparison of conventional and molecular methods of analysis. *Env. Exp. Biol.*, **9**, 9–21.

Grantina, L., Bondare, G., Janberga, A., Tabors, G., Kasparinskis, R., Nikolajeva, V. & Muiznieks, I. 2012. Monitoring seasonal changes in microbial populations of spruce forest soil of the Northern Temperate zone. *Estonian J. Ecol.*, **61**, 190–214.

Halleen, F., Schroers, H.-J., Groenewald, J. Z. & Crous, P. W. 2004. Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Stud. Mycol.*, **50**, 431–455.

Izzo, A., Nguyen, D. T. & Bruns, T. D. 2006. Spatial structure and richness of ectomycorrhizal fungi colonizing bioassay seedlings from resistant propagules in a Sierra Nevada forest: comparisons using two hosts that exhibit different seedling establishment patterns. *Mycologia*, **98**, 374–383.

Jayasinghe, B. A. T. D. & Parkinson, D. 2008. Actinomycetes as antagonists of litter decomposer fungi. *Appl. Soil Ecol.*, **38**, 109–118.

Kaldorf, M., Renker, C., Fladung, M. & Buscot, F. 2004. Characterization and spatial distribution of ectomycorrhizas colonizing aspen clones released in an experimental field. *Mycorrhiza*, **14**, 295–306.

Leger, R. J. St., May, B., Allee, L. L., Frank, D. C., Staples, R. C. & Roberts, D. W. 1992. Genetic differences in allozymes and in formation of infection structures among isolates of the entomopathogenic fungus *Metarhizium anisopliae*. *J. Invertebr. Pathol.*, **60**, 89–101.

Lumley, T. C., Gignac, L. D. & Currah, R. S. 2001. Microfungus communities of white spruce and trembling aspen logs at different stages of decay in disturbed and undisturbed sites in the boreal mixedwood region of Alberta. *Can. J. Bot.*, **79**, 76–92.

Lynch, M. D. J. & Thorn, R. G. 2006. Diversity of basidiomycetes in Michigan agricultural soils. *Appl. Environ. Microbiol.*, **72**, 7050–7056.

Makeschin, F. 1991. Influence of fast growing poplars and willows on the soil macrofauna on formerly arable land. In *Biomass for Energy, Industry and Environment: 6th E.C. Conference* (Grassi, G., Collina, A. & Zibetta, H., eds), pp. 97–103. Elsevier Applied Science, London.

Mann, L. & Tolbert, V. 2000. Soil sustainability in renewable biomass plantings. *Ambio*, **29**, 492–498.

Matthies, C., Erhard, H.-P. & Drake, H. L. 1997. Effects of pH on the comparative culturability of fungi and bacteria from acidic and less acidic forest soils. *J. Basic Microbiol.*, **37**, 335–343.

McLean, M. A. & Huhta, V. 2002. Microfungal community structure in anthropogenic birch stands in central Finland. *Biol. Fertil. Soils*, **35**, 1–12.

Menkis, A. & Burokiene, D. 2012. Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery. *For. Path.*, **42**, 79–83.

Nelson, L. E., Switzer, G. L. & Lockaby, B. G. 1987. Nutrition of *Populus deltoides* plantations during maximum production. *Forest Ecol. Manag.*, **20**, 25–41.

Nikula, S., Percy, K., Oksanen, E., Holopainen, T. & Manninen, S. 2009. Effects of elevated ozone on growth and foliar traits of European and hybrid aspen. *BER*, **14 (Suppl. A)**, 29–47.

Pečiulytė, D. & Dirginčiutė-Volodkienė, V. 2010. Effect of long-term industrial pollution on microorganisms in soil of deciduous forests situated along a pollution gradient next to a fertilizer factory. 3. Species diversity and community structure of soil fungi. *Ekologija*, **56**, 132–143.

Pedras, M. S. C. 2011. Fungal attack and cruciferous defenses: tricking plant pathogens. The biological activity of phytochemicals. *Recent Adv. Phytochem.*, **41**, 127–139.

Priha, O., Grayston, S. J., Hiukka, R., Pennanen, T. & Smolander, A. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. *Biol. Fertil. Soils*, **33**, 17–24.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org (visited 20.06.2012).

Renker, C., Otto, P., Schneider, K., Zimdars, B., Maraun, M. & Buscot, F. 2005. Oribatid mites as potential vectors for soil microfungi: study of mite-associated fungal species. *Microbial Ecol.*, **50**, 518–528.

Samson, R. A., Yilmaz, N., Houbraken, J., Spierenburg, H., Seifert, K. A., Peterson, S. W., Varga, J. & Frisvad, J. C. 2011. Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. *Stud. Mycol.*, **70**, 159–183.

Santos, M. P., Dias, L. P., Ferreira, P. C., Pasin, L. A. A. P. & Rangel, D. E. N. 2011. Cold activity and tolerance of the entomopathogenic fungus *Tolypocladium* spp. to UV-B irradiation and heat. *J. Invertebr. Pathol.*, **108**, 209–213.

Sevim, A., Demir, I., Höfte, M., Humber, R. A. & Demirbag, Z. 2010. Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. *Biocontrol*, **55**, 279–297.

Stanton, B., Eaton, J., Johnson, J., Rice, D., Schuette, B. & Moser, B. 2002. Hybrid poplar in the Pacific Northwest: the effects of market-driven management. *J. Forest.*, **100**, 28–33.

Sukarno, N., Kurihara, Y., Ilyas, M., Mangunwardoyo, W., Yuniarti, E., Sjamsuridzal, W., Park, J.-Y., Saraswati, R., Inaba, S., Widyastuti, Y., Ando, K. & Harayama, S. 2009. *Lecanicillium* and *Verticillium* species from Indonesia and Japan including three new species. *Mycoscience*, **50**, 369–379.

Torsvik, V., Sorheim, R. & Goksoyr, J. 1996. Total bacterial diversity in soil and sediment communities – a review. *J. Ind. Microbiol. Biot.*, **17**, 170–178.

Tullus, A. 2010. Tree growth and the factors affecting it in young hybrid aspen plantations. A Thesis. Estonian University of Life Sciences, Tartu.

Tullus, H., Tullus, A., Vares, A. & Kanal, A. 2007. Early growth of hybrid aspen (*Populus* × *wettsteinii* Hämet-Ahti) plantations on former agricultural lands in Estonia. *Forest Ecol. Manag.*, **245**, 118–129.

Tullus, A., Soo, T., Tullus, H., Vares, A., Kanal, A. & Roosaluste, E. 2008. Early growth and floristic diversity of hybrid aspen (*Populus* × *wettsteinii* Hämet-Ahti) plantations on a reclaimed opencast oil shale quarry in North-East Estonia. *Oil Shale*, **25**, 57–74.

Tullus, A., Tullus, H., Soo, T. & Pärn, L. 2009. Above-ground biomass characteristics of young hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) plantations on former agricultural land in Estonia. *Biomass Bioenerg.*, **33**, 1617–1625.

Tullus, A., Rytter, L., Tullus, T., Weih, M. & Tullus, H. 2012. Short-rotation forestry with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) in Northern Europe. *Scand. J. Forest Res.*, **27**, 10–29.

Watanabe, T. 2002. *Soil and Seed Fungi*. 2nd edn. CRC Press, Boca Raton.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols. A Guide to Methods and Applications* (Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J., eds), pp. 315–322. Academic Press, San Diego.

Yeates, C., Gillings, M. R., Davison, A. D., Altavilla, N. & Veal, D. A. 1998. Methods for microbial DNA extraction from soil for PCR amplification. *Biol. Proceed. Online*, **1**, 40–47.

Zaccone, C., Soler-Rovira, P., Plaza, C., Cocozza, C. & Miano, T. M. 2009. Variability in As, Ca, Cr, K, Mn, Sr, and Ti concentrations among humic acids isolated from peat using NaOH, $Na_4P_2O_7$ and NaOH + $Na_4P_2O_7$ solutions. *J. Hazard. Mater.*, **167**, 987–994.

Zeps, M., Auzenbaha, D., Gailis, A., Treimanis, A. & Grīnfelds, U. 2008. Evaluation and selection of hybrid aspen (*Populus tremuloides* × *Populus tremula*) clones. *Mežzinātne*, **18**, 19–34 (in Latvian).

Mulla mikroorganismide arvukuse ja mitmekesisuse võrdlus hariliku haava (*Populus tremula* L.) ning hübriidhaava (*Populus tremuloide*s Michx. × *P. tremula* L.) puistutes

Lelde Grantina-Ievina, Dace Saulite, Martins Zeps, Vizma Nikolajeva ja Nils Rostoks

Lühikese raieringiga majandatavaid puuliike kasutatakse maailma metsanduses üha enam. Balti regiooni kliimatingimustes on hübriidhaab (*Populus tremuloide*s Michx. × *P. tremula* L.) osutunud selleks otstarbeks sobilikuks. Lühikese raieringiga puuistandike kasvatamine endistel põllumuldadel erineb tavapõllumajandusest vähenenud maaharimise ja suurenenud toitainenõudluse poolest, mis pikas perspektiivis võib põhjustada muutusi mulla mikroobikooslustes. Käesoleva uurimuse eesmärgiks oli võrrelda mulla mikroobikooslusi hübriidhaava ja hariliku haava (*P. tremula* L.) puistutes neljal proovialal, kus haabade vanus jäi vahemikku 10 kuni 46 aastat. Mulla mikroobikoosluste arvukuse ja mitmekesisuse hindamiseks kasutati nende loendamist (plaadikülv kolmel erineval tardsöötmel) ning molekulaarseid meetodeid (PCR, ARDRA, seene isolaatide molekulaarne identifitseerimine). Tulemused näitasid, et hübriidhaava pikaaegse kasvatamise tagajärjel väheneb kultiveeritavate bakterite arvukus. Kultiveeritavate niitjate seente arvukus oli statistiliselt usaldusväärselt suurenenud vaid ühe hübriidhaava katseala mullas sügavusel 16–30 cm ja ainult ühel mikrobioloogilisel söötmel. Seda kinnitas mainitud katsealal ka molekulaarsete meetoditega määratud seente mitmekesisus, mida hinnati Shannon-Weaveri mitmekesisuse indeksiga. Teisi mulla mikroobikoosluse tunnuseid (pärmi ja maltoosi tarvitavate bakterite arvukus virdeagaril, pärmide ning niitjate seente arvukus Rose-Bengali tardsöötmel, kogu mullast eraldatud DNA, molekulaarbioloogiliste meetoditega leitud seente ja bakterite mitmekesisus ning niitjate seente liigiline koosseis) ei olnud hübriidhaabade kasv usaldusväärselt mõjutanud. Määratud niitjad seened esindasid järgmisi perekondi: *Acremonium*, *Exophiala*, *Geomyces*, *Gibellulopsis*, *Gibberella*, *Hypocrea*/*Trichoderma*, *Leptosphaeria*, *Metarhizium*, *Mortierella*, *Nectria*, *Paecilomyces*, *Penicillium*, *Trichosporon* ja teised. Peamise järeldusena leiti, et hübriidhaava kasvatamine lühikese raieringiga majandatava lehtpuuna Balti regioonis ei mõjuta märgatavalt saprofüütsete mulla mikroorganismide ohtrust ja mitmekesisust.