Features of saprophytic soil microorganism communities in conifer stands with or without *Heterobasidion annosum sensu lato* infection: a special emphasis on *Penicillium* spp.

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Abstract

The aim of the study was to characterize soil microbial communities of nine conifer (*Pinus sylvestris, Picea abies*) stands mainly on podzolic soils, e.a., seven healthy stands (28 to 160 years) and two stands infected with root rot fungus *Heterobasidion annosum s.l.* (50 and 56 years). The studied microbial variables differed for healthy conifer stands on automorphic soils vs. infected conifer stands on semihydromorphic soils, and differed depending on forest age. Automorphic soils had significantly higher numbers of filamentous fungi in the upper soil layert han in the deeper layer. In semihydromorphic soils both analyzed soil layers had similar numbers of fungal colony-forming units. The pine stand had significantly lower yeast and maltose-utilizing bacteria density in the 11 to 30 cm soil layer. Sampling plots on semihydromorphic soils including infected stands lacked species of the *Mortierella* genus or had them in low percentage, but had increased percentage of colony-forming units of *Umbelopsis* genus. Semihydromorphic soils had decreased proportion of colony-forming units of *Penicillium* spp. and almost no *Trichoderma* spp. Molecular biology analysis showed that the average fungal diversity indice *H'* in automorphic soils was significantly higher than in semihydromorphic soils. The highest fungal diversity in respect to represented genera was in the oldest sampling plot (16 genera). Antagonism assay of 52 *Penicillium* spp. isolates showed reduced growth rate of *H. annosum s.l.* but the antagonistic effect was not as strong as it has been reported for *Trichoderma* spp.

Key words: antagonism assay, *Heterobasidion annosum, Mortierella, Penicillium*, soil microbial diversity, *Umbelopsis*.
Abbreviations: ARDRA, amplified ribosomal DNA restriction analysis; CFU, colony-forming units; MEA, malt extract agar; MSCL, Microbial Strain Collection of Latvia; PCR, polymerase chain reaction; *s.l., sensu lato; s.s., sensu stricto*.

Introduction

Soil in general is a very heterogeneous environment and the components of the solid fractions in soil such as sand, silt, clay, and organic matter provide variable microhabitats for the soil microorganisms (Garbeva at al. 2004). The most important soil parameters are particle size, pH, cation exchange capacity and organic matter content. These parameters can affect microbial community structure either directly by providing a specific habitat that selects specific microorganisms, or indirectly by affecting plant root functioning and exudation in a soil-specific manner. In some situations the soil and in others plant type is the determining factor affecting the soil microbial community (Garbeva et al. 2004).

Forest soils differ from agricultural soils by their vertical stratification due to the forest litter accumulation that forms the humic horizon, and saprotrophic soil fungi are one of the main groups of soil microorganisms responsible for litter degradation (Zeller et al. 2007; Baldrian et al. 2010; Allison, Treseder 2011). Their extracellular enzymes are active not only in the litter layer but also in the deeper soil horizons (Baldrian et al. 2010).

It is reported that several properties of site and soil also affect the occurrence and severity of the damage of such serious tree root pathogen like *Heterobasidion annosum sensu lato* (s.l.), which is widely found in Latvia (Grantina et al. 2000) and in other countries of the boreo-nemoral region. In general, high risk of *H. annosum* damage has been associated with environmental conditions and climate zones that determine soil properties and soil types. According to Pratt et al. (2002), *H. annosum* hazard increases with increase of site fertility (mainly on well drained calcareous soils with high pH value). However, hazard decreases with increase of soil moisture conditions (surface water gley and groundwater gley features) and accumulation of peat. According to Kaarna-Vuorinen (2000) the relative frequency of butt rot is higher on drained peatland sites than on undrained peatland sites. The incidence of disease is also reported to be higher in mineral soils with a fluctuating groundwater table (Thor 2005). In addition, ditching of mineral soil sites increases the risk of *H. annosum* butt rot damage, possibly because the root systems suffer from occasional drought (Korhonen et al. 1998).

High incidence of root rot is correlated with lack of

antagonistic fungi like *Trichoderma* spp. and *Penicillum* spp. (Korhonen, Stenlid 1998; Stenlid, Redfern, 1998). Besides several species of *Trichoderma* genus that have been screened for their antagonism against *H. annosum s.l.* (Nikolajeva et al. 2012) other soil microfungi have been reported to show antagonistic properties against *H. annosum s.l.* For example, several fungal isolates from 25 to 45 years old spruce stands from *Myrtillosa mel., Hylocomiosa, Oxalidosa* forest types have shown antagonistic abilities against the S and P group of *H. annosum s.l. in vitro*. These were members of the genera *Aureobasidium* (only against S group), *Geotrichum*, some isolates from the genus *Mortierella, Mucor, Mycogone, Oidiodendron, Penicillium, Rhizopus, Trichoderma, Trichosporon*, and partly *Verticillium* (Arhipova et al. 2008). A similar spectrum of fungal genera with antagonistic properties were found in a later investigation of this research group on the microflora of the spruce rhizosphere in stands established on former agricultural lands and forest lands (Gaitnieks et al. 2009). From the forest soils of Scots pine (*Pinus sylvestris*) stands in Poland also several fungi have been isolated showing antagonistic properties against the *H. annosum* P group – *Absidia glauca, Gliocladium viride, Mortierella isabellina* (part of isolates), *Oidiodendron tenuissimum, Penicillium daleae, Penicillium janczewskii, Penicillium spinulosum, Penicillium purpurogenum, Penicillium waksmanii, Penicillium decumbens, Rhizopus oryzae, Thysanophora penicillioides, Trichoderma koningii, Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride,* and *Verticillium* sp. (Manka et al. 2006).

The factors by which soils can be suppressive to different soil-borne pathogens can involve biotic (soil microflora) and/or abiotic elements (soil physical and chemical properties), and their impact may be different on various pathogens. It has been suggested that the main agents in soil suppressiveness are microbial (Garbeva et al. 2004).

In this investigation microbial communities in soils of healthy forest stands will be compared with those in soils of forest stands that are infected with pathogenic fungus *H. annosum s.l.* The aim of this investigation was to create baseline information about particular characteristics of soil microbial communities depending from soil group and forest age and to evaluate the antagonistic abilities of particular soil filamentous fungi against *H. annosum s.l.* The objectives of the study were to characterize and compare soil microbial communities using following methods: enumeration of cultivable microorganisms; determination of typical fungal genera and species using molecular biology methods; extraction of total soil DNA; polymerase chain reaction (PCR) followed by amplified ribosomal DNA restriction analysis (ARDRA) and calculations of Shannon-Weaver diversity indices; estimation of antagonistic abilities of 52 *Penicillium* spp. isolates from agricultural soil, former agricultural soil and forest soil against two isolates of *H. annosum sensu stricto* (*s.s.*), one isolate of *H. annosum s.l.* and two isolates of H*eterobasidion parviporum*.

Materials and methods

Sampling plots, soil and wood sampling

Sampling plots were located in the Ropaži district of Latvia. These forest stands are under the management of Riga Forests Ltd. Characteristics of each sampling plot are described in Table 1. From all forest stands 20 wood samples were obtained with increment borer and placed in sterile glass test-tubes. Where present fruiting bodies of *H. annosum s.l.* were sampled (sampling plot Spruce 50).

Soil samples (500 g) were taken in two depths (0 to 10 cm and 11 to 30 cm) using a modified method described by Luis et al. (2005). Sampling plots were circular with three transects along which three samples in each depth

Table 1. Sampling plots, their characterization and sampling time. Forest types are according to the classification of Buss (1997). [a], Automorphic soils – soils without gleyic or gley horizons. The formations of gley can be observed only as separate pathces under the organic horizon and/or as obstructions in gaps and tunnels of earthworms. [b], Semihydromorphic soils – seen only with standing water or medium deep groundwater (Karklins 2008)

Sampling plot		Sampling date	Forest age (years)	Soil type according to Latvian soil classification (Karklins 2008)	Soil group according to FAO WRB 2006
Healthy forest stands					
Pine 80	*Myrtillosa*	June 2010	80	Typic podzol[a]	Folic Arenosols
Spruce 37	*Oxalidosa*	August 2010	37	Illuvial humic podzol[a]	Folic Albic Podzols
Spruce 47	*Oxalidosa*	July 2010	47	Stagnogley sod-podzolic soil[b]	Folic Gleyic Calcic Luvic Planosols
Spruce 28	*Hylocomiosa*	June 2010	28	Illuvial humus podzol[a]	Podzols
Spruce 62	*Hylocomiosa*	June 2010	62	Typic podzol[a]	Folic Albic Podzols
Spruce 141	*Hylocomiosa*	August 2010	141	Typic podzol[a]	Folic Podzol
Spruce 160	*Vacciniosa*	June 2010	160	Sod-podzolic soil[a]	Folic Arenosols
Infected forest stands					
Spruce 50	*Oxalidosa*	July 2010	50	Humi-podzolic gley soil[b]	Rustic Folic Endogleyic Podzols
Spruce 56	*Hylocomiosa*	July 2010	56	Humi-podzolic gleyic soil[b]	Cutanic Albic Gleyic Luvisols

were taken, at 0.3 m, 1.0 m and 3.0 m from the centre. Samples were placed in sterile plastic bags (Nasco WHIRL-PAK), stored at 4 °C for a few days until the estimation of cultivable microorganisms and then were stored at –20 °C. In total 54 soil samples were analyzed.

Physical and chemical analysis of soil

Soil moisture content was determined according to the ISO 11465 standard method for every soil sample. The pH of soil samples was measured in distilled water according to the ISO 10390 method. Soil physical and chemical analysis was performed in the Laboratory of Forest Regeneration and Establishment Group of 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; and 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). From every plot four soil samples in three chemical replicates were analyzed.

Estimation of number of microorganisms and determination of dominant fungal genera

These parameters were determined in every soil sample in three replicates. In order to estimate the amount of colony forming units (CFU) of filamentous fungi, bacteria and yeasts soil sample decimal dilutions were prepared using sterile ddH_2O. Agarised malt extract (MEA, Biolife, Italy, 30 g L^{-1}, pH 5.4 ± 0.2) was used as growing medium, as it is favorable for filamentous fungi, yeasts and particular groups of bacteria, for example, actinobacteria. Further in the text all these groups together are referred to as cultivable microorganisms. Incubation time was three days for the enumeration of bacterial CFU and five days for the enumeration of fungal CFU, and temperature of incubation was 20 ± 2 °C. Fungal genera were determined according to morphological characteristics and light microscopy results using keys.

Detection of root rot fungus in wood samples

In the laboratory, collected pieces of wood were placed on the MEA (15 g L^{-1}) and incubated at room temperature for 1 to 2 weeks. The mycelia of root rot fungus was identified microscopically and placed on new Petri dishes with MEA. Forest stands from which no isolate of *Heterobasidion* sp. was obtained and no fruiting bodies were detected on the site, and also trees lacked any signs of infection, were further considered as healthy forest stands.

Extraction of total soil DNA, PCR and ARDRA

Total soil DNA was extracted using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc.). Samples were homogenized using a horizontal Mixer Mill Type MM 301 (Retsch) at maximal speed 30 Hz (1800 oscillations min^{-1}). The amount and purity of the DNA was detected spectrophotometricaly using Ultrospec 3100 Pro (Amersham Biosciences). In PCR we amplified the fungal nuclear ribosomal DNA region that contains two internal transcribed spacers and the 5.8S rRNA gene (ITS1-5.8S-ITS2) with primers ITS1F (Gardes, Bruns 1993) and ITS4 (White et al. 1990). These primers amplify the ITS1-5.8S-ITS2 region of *Ascomycota, Basidiomycota* and *Zygomycota* fungi. The PCR conditions and ARDRA methodology was used as described previously (Grantina et al. 2012; Grantina-Ievina et al. 2012). For ARDRA analysis the restriction endonuclease *Bsu*RI (Chabrerie et al. 2003) was used. For the calculation of the Shannon-Weaver diversity index (*H'*) the following equation was used:

$$H' = -\Sigma\, p_j \log_2 p_j,$$

where p_j is a relative intensity of individual band (Gabor et al. 2003).

Sequencing of ribosomal DNA

In total, 59 isolates (53 sporulating and six white sterile mycelia, not sporulating while kept at 4 °C for several months) representing dominant filamentous fungi were isolated from the plates used for the enumeration of cultivable filamentous fungi and subcultured on MEA. Genomic DNA from approximately 0.25 g of mycelia was extracted using a PowerSoil™ DNA Isolation Kit. Extracted DNA was amplified by PCR with primers ITS4 and ITS1F. Amplified DNA fragments from fungal isolates were sequenced in the CBS KNAW Fungal biodiversity centre, Utrecht, the Netherlands in the frame of project EMbaRC. After PCR, 5 µL of amplified products were subjected to a sequencing protocol with a BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems, USA) with both primers. Double stranded sequences of PCR amplicons were assembled using Staden Package 1.6.0. Homology search in the National Centre for Biotechnology Information (NCBI) GenBank nucleotide database was conducted using the Basic Local Alignment Search Tool.

Antagonism assay

In total, 52 *Penicillium* spp. isolates (Apendix 1) from agricultural soil, former agricultural soil (aspen stands) and forest soil including eight isolates from the present study and two isolates from spruce stands infected with

H. parviporum from the previous investigation (Grantina et al. 2012) were used in antagonism tests on 3 % MEA against two isolates of *H. annosum s.s.* (strain number in Microbial Strain Collection of Latvia MSCL 1020, MSCL 1021), one isolate from the sampling plot S56 *H. annosum s.l.* and two isolates of *H. parviporum* (MSCL 980, MSCL 981). Incubation temperature was 20 ± 2 °C. Each pairing with root rot isolates was performed twice. Petri dishes were checked and colonies measured three times or more until complete overgrowth of the dish had occurred (approximately 30 days). The efficiency of *Penicillium* in suppressing radial growth was calculated using growth measurements after 7 to 9 days of incubation as follows:

$$(C - T) / C \times 100,$$

where C is radial growth measurement of the pathogen in the control and T is radial growth of the pathogen in the presence of *Penicillium* (Asran-Amal et al. 2005).

Statistical analysis

For statistical analysis the *F*-test, *t*-test ($\alpha = 0.05$), and correlation analysis with Excel function CORREL were performed. Multiple regression analysis was done with the R package (R Development Core Team 2009). Due to co-linearity of total carbon content values and calcium, magnesium and potassium values, C/N ratios were used in the regression models instead of total carbon content and nitrogen content.

Results and discussion

Physical and chemical characterization of soil

Data on element content, soil pH and soil moisture content are listed in Table 2. Soil pH_{H2O} was similar in all sampling plots and ranged from 3.71 to 5.08. The concentration of all analyzed elements significantly differed between both analyzed soil depths. The concentrations of several elements were significantly higher in semihydromorphic soils. For example, at depth 0 to 10 cm, concentrations of calcium ($F = 2.00$, $p = 0.02$), magnesium ($F = 5.73$, $p = 0.008$), potassium ($F = 2.81$, $p = 0.02$), carbon ($F = 8.57$, $p = 0.04$) and nitrogen ($F = 3.54$, $p = 0.042$) were significantly higher. At depth 11 to 30 cm, significantly higher levels of magnesium ($F = 2.62$, $p = 0.02$) and potassium ($F = 1.97$, $p = 0.003$) were observed.

The average soil moisture content of semihydromorphic soils was higher than of authomorphic soils, 54.07 ± 15.19% vs. 37.10 ± 21.52%, although this difference was not statistically significant.

Regarding soil texture, in all sampling plots the 0 to 10 cm sample contained only organic matter. At depth of 11 to 30 cm, the soil texture class was sand in sampling plots Pine 80, Spruce 62 and Spruce 160 and loamy soil in sampling plot Spruce 56. In other sampling plots the soil in this depth contained only organic matter.

Presence of root rot fungus in wood samples

Mycelium of *H. annosum s.l.* was isolated only from wood samples of sampling plot S56. Infection of root rot was observed only in two sampling plots, S50 and S56.

Amount of cultivable microorganisms and dominant fungal genera

The average numbers of CFU of filamentous fungi are given in Fig. 1. There was a clear difference between the two soil groups, automorphic and semihydromorphic soils. In sampling plots with automorphic soils the numbers of fungal CFU significantlt differed between soil depths. In the upper soil layer there were more filamentous fungi. In the sampling plots with semihydromorphic soils, which include two infected forest stands, the soil layers had similar number of fungal CFU. There were no differences between the soil of pine stand and spruce stands.

Significant differences in number of CFU of yeasts and maltose utilizing bacteria (Fig. 2) between dryer and moister soil types were not observed. In almost all sampling plots (with exception plot Spruce 50) there were significantly higher numbers of CFU of yeasts and maltose utilizing bacteria in the upper soil horizon. The pine stand had significantly lower number of yeasts and maltose utilizing bacteria, in comparison with spruce stands, in the deepest soil layer (11 to 30 cm; $F = 21.89$, $p < 0.001$), which can be explained by higher pH value (5.08).

The decrease of the abundance of microorganisms with depth has been previously reported, for example, in two soil profiles covered by annual grasses as vegetation in a Mediterranean climate (Fierer et al. 2003), or in forest soil profiles in Italy (Agnelli et al. 2004) and in deciduous woodland in UK (Castellazzi et al. 2004).

Regarding observed differences between the two soil moisture classes (automorphic and semihydromorphic soils), it has been reported that hydromorphic soils have higher yeast abundance than mineral soils (Polyakova et al. 2001) and that soddy-gley hydromorphic forest soils have high numbers of hydrolytic bacteria in comparison with peaty-podzolic and peat soils (Dobrovolskaya et al. 2000).

Abundances of the main fungal genera and sterile mycelia are given in Table 3. There were clear differences in the composition of the dominant fungal genera between automorphic and semihydromorphic soil classes. Sampling plots with semihydromorphic soils (Spruce 47, Spruce 50, Spruce 56) including infected stands, lacked species of *Mortierella* genus or their relative abundance of CFU was low (in average 0.67 %), but had higher percentage of CFU of the *Umbelopsis* genus (in average 40.66 %). These two genera are close relatives, and formerly the genus *Umbelopsis* with coloured colonies belonged to one section of the *Mortierella* genus. They both produce arachidonic acid, whitch acts as a suppressive agent of plant pathogenic fungi (Eroshin, Dedyukhina 2002; Fakas et al. 2009). *Mortierella* species are known as phosphate solubilizers

Table 2. Element concentration, C/N ratio, the content of humic acids, soil pH_{H2O} and soil moisture content in two depths in sampling plots. Data are means ± SD. *, significant (n = 3) differences between the soil samples from the two depths

Plot / Depth (cm)	Ca_{total} (g kg^{-1})	Mg_{total} (g kg^{-1})	K_{total} (g kg^{-1})	C_{total} (g kg^{-1})	P_{total} (g kg^{-1})	N_{total} (g kg^{-1})	C/N ratio	Humic acids (g kg^{-1})	pH_{H2O}	Soil moisture (%)
Pine 80										
0-10	1.32 ± 0.089*	0.56 ± 0.038*	0.44 ± 0.020*	155.8 ± 0.6*	0.11 ± 0.00*	3.9 ± 0.10*	40	70.9 ± 1.72*	4.02	31.29±3.03*
11-30	0.03 ± 0.002*	0.28 ± 0.019*	0.20 ± 0.009*	10.6 ± 0.1*	0.15 ± 0.01*	0.2 ± 0.00*	48	17.5 ± 0.09*	5.08	6.57±1.14*
Spruce 37										
0-10	1.81 ± 0.122*	0.50 ± 0.034*	0.33 ± 0.015*	247.0 ± 1.1*	0.16 ± 0.01*	6.7 ± 0.40*	37	95.2 ± 1.93*	4.01	47.83±2.07*
11-30	1.49 ± 0.101*	0.24 ± 0.016*	0.26 ± 0.012*	151.2 ± 4.0*	0.07 ± 0.00*	2.9 ± 0.40*	53	60.8 ± 1.43*	4.04	23.64±1.94*
Spruce 47										
0-10	4.54 ± 0.307*	1.08 ± 0.073*	0.80 ± 0.036*	439.4 ± 9.8*	0.29 ± 0.00*	13.3 ± 0.10*	33	96.4 ± 2.31*	4.01	52.40±4.58*
11-30	0.34 ± 0.023*	0.50 ± 0.034*	0.59 ± 0.027*	98.3 ± 3.0*	0.11 ± 0.01*	2.9 ± 0.00*	34	65.7 ± 0.03*	3.93	32.68±2.45*
Spruce 28										
0-10	2.26 ± 0.153*	0.89 ± 0.060*	0.70 ± 0.032*	434.1 ± 14.4*	0.31 ± 0.04*	14.0 ± 0.10*	48	135.4 ± 23.76*	3.92	71.68±5.48*
11-30	3.00 ± 0.203*	0.47 ± 0.032*	0.36 ± 0.016*	163.4 ± 1.8*	0.11 ± 0.01*	3.4 ± 0.20*	31	94.7 ± 0.68*	3.81	36.65±2.97*
Spruce 62										
0-10	3.32 ± 0.224*	0.83 ± 0.056*	0.50 ± 0.023*	427.7 ± 1.1*	0.28 ± 0.01*	13.8 ± 0.10*	31	89.3 ± 8.20*	3.94	60.00±3.85*
11-30	0.78 ± 0.053*	0.19 ± 0.013*	0.14 ± 0.006*	40.4 ± 0.3*	0.02 ± 0.00*	0.93 ± 0.10*	45	18. ± 2.94*	4.02	16.15±7.02*
Spruce 141										
0-10	2.22 ± 0.150*	0.62 ± 0.042*	0.48 ± 0.022*	301.5 ± 4.0*	0.21 ± 0.02*	10.8 ± 0.10*	28	106.2 ± 5.00*	4.22	62.07±3.86*
11-30	0.90 ± 0.061*	0.20 ± 0.014*	0.17 ± 0.008*	109.8 ± 6.3*	0.05 ± 0.01*	2.4 ± 0.30*	45	41.9 ± 2.19*	4.15	23.70±3.85*
Spruce 160										
0-10	0.58 ± 0.039*	0.72 ± 0.049*	0.58 ± 0.026*	291.8 ± 6.9*	0.18 ± 0.01*	8.1 ± 0.30*	36	87.9 ± 16.42*	3.72	53.25±4.50*
11-30	0.00 ± 0.000*	0.22 ± 0.015*	0.21 ± 0.010*	26.5 ± 0.9*	0.05 ± 0.01*	0.8 ± 0.10*	34	17.6 ± 1.07*	3.97	12.40±1.98*
Spruce 50										
0-10	3.47 ± 0.234*	0.96 ± 0.065*	0.65 ± 0.030*	459.5 ± 3.3*	0.29 ± 0.02*	17.2 ± 0.20*	27	86.4 ± 1.14*	4.18	67.60±4.10
11-30	2.17 ± 0.147*	0.73 ± 0.049*	0.54 ± 0.025*	310.8 ± 4.6*	0.19 ± 0.00*	10.0 ± 0.20*	31	126.5 ± 0.08*	3.95	62.76±4.33
Spruce 56										
0-10	3.34 ± 0.226*	1.06 ± 0.072*	0.73 ± 0.033*	510.4 ± 2.8*	0.33 ± 0.02*	16.8 ± 0.40*	30	105.6 ± 6.12*	4.03	56.19±4.39*
11-30	0.54 ± 0.036*	0.40 ± 0.027*	0.38 ± 0.017*	100.7 ± 1.1*	0.11 ± 0.00*	3.3 ± 0.20*	30	71.4 ± 0.75*	3.88	43.81±4.85*

and soil inoculation with propagules of these fungi has been shown to increase arbuscular mycorrhization of *Kostelelzkya virginica* in soil with increased salinity (Zhang et al. 2011).

Semihydromorphic soils also had significantly lower proportion of CFU of *Penicillium* spp. than in automorphic soils (13.95 ± 9.50 vs. 32.26 ± 12.12 %; $F = 1.64$, $p = 0.005$). In the soil of infected stands *Penicillium* spp. were less abundant than in healthy stands (12.09 ± 10.84 vs. 30.18 ± 12.51%; $F = 1.33$, $p = 0.02$). Semihydromorphic soils and soils from infected stands had practically no *Trichoderma* spp. (only the Spruce 56 plot had *Trichoderma* spp. 0.11% from all cultivable filamentous fungi). The incidence of root rot is associated with the lack of antagonistic fungi like *Trichoderma* and *Penicillum* spp. (Korhonen, Stenlid 1998), as also observed in our study. In our previous investigation where two infected spruce stands on authomorphic soils were analyzed, in the active vegetation period (from May to September), the relative number of *Penicillium* spp. was 33.20 to 38.39%, *Trichoderma* spp. 0.86 to 2.43%, and *Mortierella* spp. 9.26 to 13.63%, of all filamentous fungi in the soils (Grantina et al. 2012). In another investigation in Latvia where soil microbiology of twenty six 25- to 45-year-old spruce stands (forest types *Myrtillosa mel., Hylocomiosa, Oxalidosa*; soil types were not identified) were analyzed, soils of infected stands had lower abundance of *Trichoderma* spp., higher abundance of *Penicillium* spp., presence of several isolates of *Mortierella* also in soils of infected stands, and *Penicillium* showed antagonistic abilities against isolates of *H. annosum* S and P group in vitro (Arhipova et al. 2008).

Forest soil in the oldest stand Spruce 160 at depth of 11 to 30 cm had the highest number of represented fungal genera (10): *Aspergillus, Botrytis, Cladosporium, Humicola, Mortierella, Mucor, Penicillium, Trichoderma, Umbelopsis, Verticillium* and sterile mycelia. In other sampling plots, three to five genera and sterile mycelia were represented.

Total soil DNA

The amount of DNA isolated by a PowerSoil™ DNA

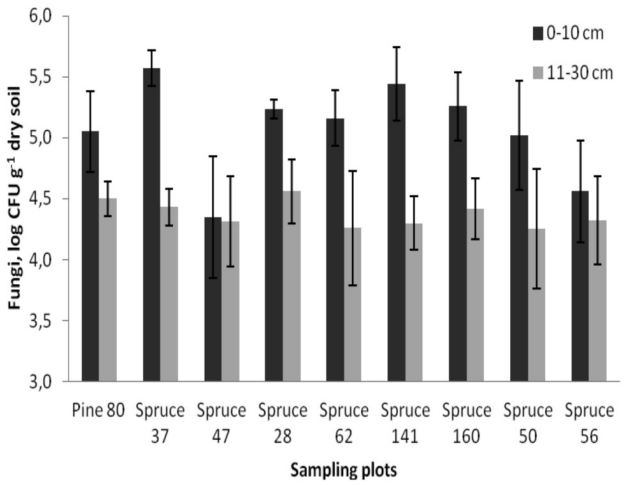

Fig. 1. Number of filamentous fungi in the studied soils (± SD; n = 9).

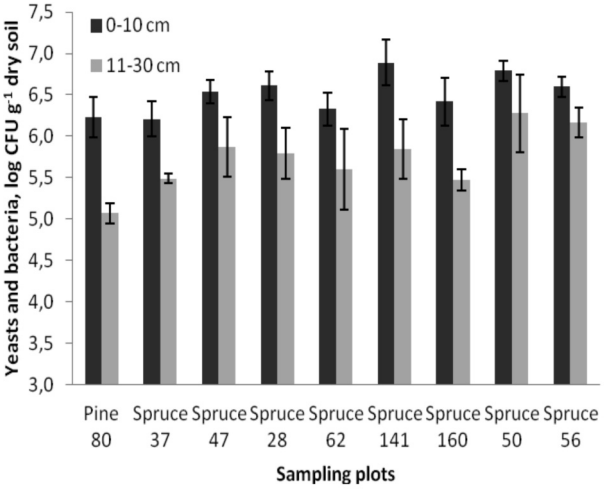

Fig. 2. Number of yeasts and maltose-utilizing bacteria in the studied soils (± SD; n = 9).

Isolation Kit ranged from 10.65 μg g^{-1} in deeper soil layers to 119.46 μg g^{-1} of dry soil in the upper soil horizon (Fig. 3). There were no significant differences between automorphic and semihydromorphic soils or between pine and spruce stands in the amount of total soil DNA but in all sampling plots the upper soil layer had significantly higher amounts of total soil DNA, as found elsewhere (Agnelli et al. 2004).

Fungal diversity

The estimated Shannon-Weaver diversity indices of *Ascomycota, Basidiomycota* and *Zygomycota* fungi using ARDRA are given in Fig. 4. There were no significant differences between pine and spruce stands in fungal diversity but the average fungal diversity *H'* in automorphic soils was significantly higher than in semihydromorphic soils, including infected stands (2.73 ± 0.33 vs. 2.18 ± 0.51; $F = 1.39$, $p = 0.009$). There were no significant differences in diversity between soil depth, with the exception of sampling plot Spruce 50 which had higher fungal diversity indices in the deepest soil layer. Similarly, it was found that in loblolly pine (*Pinus taeda*) forest mycorrhizal species predominated deeper in the soil profile while saprophytic species were more common in the litter layer (O' Brien et al. 2005).

Taxonomic analysis of soil fungal populations

A list of fungal genera determined according to morphological characters or DNA sequencing is given in Appendix 2. Of 59 sequenced isolates (28 species), 69.49% were *Ascomycota* (representing 17 genera), 28.81% were *Zygomycota* (three genera) and only 1.69% were *Basidiomycota* (one species *Thanatephorus cucumeris*). The low proportion of *Basidiomycota* is likely due to microbiological medium used (MEA) for isolation. In other investigations, it is known that *Basidiomycota* fungi dominate among all soil fungi in forests (Buee et al. 2009). In our previous investigations of forest soils using MEA, we isolated a few basidiomycetes like *Fomitopsis pinicola, Gloeophyllum sepiarium* (Grantina et al. 2010), and *Trichosporon porosum* (Grantina et al. 2012; Grantina-Ievina et al. in press), which represented only 1.19 to 1.36% of the total number of isolated and sequenced fungi. The best methods for studying soil basidiomycetes are hyphal isolation (Warcup 1951), collection of fruit-bodies and analysis of soil DNA (Luis et al. 2004).

The most abundant genus in all sampling plots and at both sampling depths was *Penicillium*. The following species were identified: *Penicillium canescens, Penicillium citreonigrum, Penicillium glaucoalbidum, Penicillium janthinellum, P. spinulosum* and *Penicillium thomii*. Both *P. citreonigrum* and *P. canescens* have been described in the literature as soil saprotrophic microfungi isolated from oak (*Quercus petraea*) forest with an acidic Cambisol in Czech Republic (Baldrian et al. 2011). *P. janthinellum* has been isolated from soil of an European aspen (*Populus tremula* L.) stand in Latvia (Grantina-Ievina et al. 2012). *P. spinulosum* has been isolated from an illuvial humus podzol soil of a spruce stand infected with *Heterobasidion parviporum* (Grantina et al. 2012). *P. canescens* was isolated from the soil of European aspen and hybrid aspen stands (*Populus tremuloides* Michx. × *Populus tremula* L.) (Grantina-Ievina et al. 2012) and from organic agriculture fields (Grantina et al. 2011). *P. glaucoalbidum,* which was isolated from the sampling plot Spruce 37, is known to be decomposer of spruce needle litter (Koukol et al. 2008).

The next most common genus isolated from all sampling plots except Spruce 50 was *Trichoderma* / *Hypocrea*. *Trichoderma asperellum, Trichoderma viride, Hypocrea pachybasioides* and *Hypocrea viridescens* (teleomorph of *Trichoderma viridescens*) have been isolated from spruce stands infected with *H. parviporum* (Grantina et al. 2012) and latter two species have been detected also in soil of aspen stands (Grantina-Ievina et al. 2012). *Hypocrea citrina* (teleomorph of *Trichoderma lacteum*) from the sampling

Table 3. Relative CFU of main fungal genera and sterile mycelia in two soil depths. Other genera: *Verticillium*, *Humicola* and *Cladosporium* spp.

Plot	Depth (cm)	*Mortierella* spp. (%)	*Penicillium* spp. (%)	*Trichoderma* spp. (%)	*Mucor* spp. (%)	*Aspergillus* spp. (%)	*Botrytis* spp. (%)	*Umbelopsis* spp. (%)	Other genera (%)	Sterile mycelia (%)
Pine 80	0-10	13.19	38.57	15.20	0.07	0.00	0.00	0.00	6.59	26.37
	11-30	0.16	31.80	1.82	0.00	0.78	0.00	0.00	0.78	64.67
Spruce 37	0-10	30.45	31.12	10.45	7.48	0.00	0.00	9.80	0.00	10.71
	11-30	21.07	30.77	16.87	7.75	0.00	0.00	1.76	0.00	21.78
Spruce 47	0-10	0.00	22.63	1.06	0.11	0.00	0.00	41.44	0.00	34.76
	11-30	0.00	12.23	1.95	0.00	0.00	0.00	22.08	0.00	63.73
Spruce 28	0-10	12.07	36.20	37.19	0.63	0.00	0.00	0.49	0.00	13.41
	11-30	46.11	14.85	7.76	1.23	0.00	0.00	0.18	0.00	29.88
Spruce 62	0-10	14.26	47.00	7.23	1.10	0.00	0.00	8.08	0.00	22.34
	11-30	14.53	15.68	4.81	0.03	0.00	0.00	0.11	0.00	64.83
Spruce 141	0-10	3.71	41.89	29.18	3.14	0.00	0.28	4.81	0.00	16.98
	11-30	53.30	19.19	6.61	0.03	0.00	0.00	1.69	0.00	19.19
Spruce 160	0-10	19.38	53.76	4.02	4.18	0.00	0.00	7.31	0.00	11.34
	11-30	1.08	26.31	5.17	0.16	10,38	5.64	1.41	25.37	24.48
Spruce 50	0-10	0.00	10.06	0.00	0.92	0.00	0.00	83.49	0.00	5.53
	11-30	0.00	10.80	0.00	0.03	0.00	0.00	50.20	0.00	38.97
Spruce 56	0-10	3.72	26.80	0.11	0.00	0.00	0.00	42.96	0.00	26.41
	11-30	0.31	0.65	3.12	0.00	0.00	0.00	3.77	0.00	92.14

plot Spruce 56 was isolated also from leaf litter and rich soils in Europe, Japan and North America (Overton et al. 2006).

Several isolates from the genus *Lecythophora* (*Lecythophora mutabilis* and *Lecythophora* sp.) were isolated from bulk soil in sampling plots Pine 80, Pruce 62 and Spruce 56. *L. mutabilis* has been detected in the rhizosphere of prairie grass *Andropogon gerardii* (Jumponen 2011), isolated as a root endophyte of *Betula papyrifera* and *Abies balsamea* in boreal forests in eastern Canada (Kernaghan, Patriquin 2011), and from roots of *Pinus sylvestris* seedlings (Menkis, Vasaitis 2011). *L. mutabilis* has been isolated from the illuvial humus podzol soil of spruce stand infected with *Heterobasidion parviporum* (Grantina et al. 2012).

The entomopathogenic species *Beauveria bassiana* was isolated from two sampling plots Spruce 37 and Spruce 141. This species has been isolated from sod-podzolic soil of a spruce stand infected with *H. parviporum* (Grantina et al. 2012). In other stduies this species has been isolated from roots of healthy pine *Pinus sylvestris* trees but not from trees in decline (Lygis et al. 2004). Another entomopathogenic species *Tolypocladium cylindrosporum* (synonym *Beauveria*

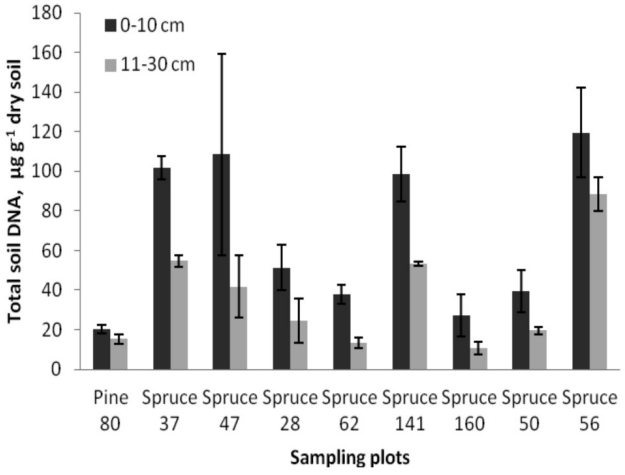

Fig. 3. The amount of total soil DNA in sampling plots at two depths (± SD; n = 3).

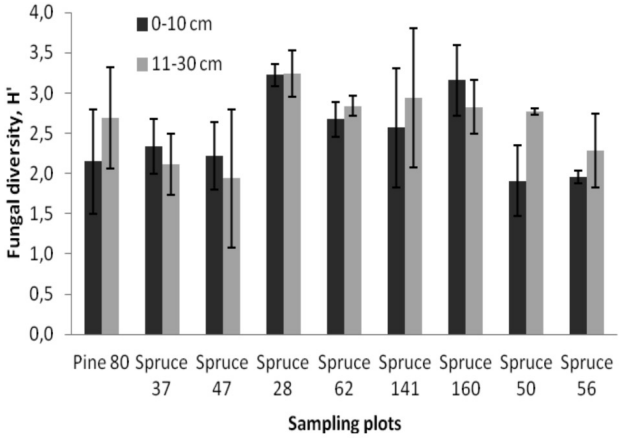

Fig. 4. The average fungal diversity *H'* at two soil depths in sampling plots (± SD; n = 3).

cylindrospora) was isolated from soil of Spruce 28 and Spruce 160. In a study conducted in Šumava National Park in Czech Republic this species was isolated from soil under spruce colonized with *Ips typographus* (Landa et al. 2001).

Pochonia bulbillosa was isolated from three sampling plots, Spruce 37, Spruce 62 and Spruce 141. Previously this species has been isolated from illuvial humus podzol soil of a spruce stand infected with *H. parviporum* (Grantina et al. 2012), from peatlands (Thormann et al. 2004) and as a decomposer of spruce wood (Thormann 2006).

Stilbella species were isolated from three sampling plots, Spruce 37, Spruce 141 and Spruce 160. *Stilbella byssiseda* (Spruce 141) has been detected in soil of *Salix herbacea* in the Austrian Central Alps (Oberkofler, Peintner 2008). This fungus is known to colonize the fructifications of myxomycetes (Rogerson, Stephenson 1993).

The highest fungal diversity in the terms of the number of represented genera (16 genera) was in the oldest sampling plot Spruce 160. Two *Mucorales* were identified as *Absidia psychrophilia* and *Ambomucor seriatoinflatus,* and four isolates with sterile mycelia as *Chalara longipes, Thanatephorus cucumeris, Stilbella* sp. and *Tolypocladium cylindrosporum*. In other sampling plots, the number of genera ranged from three to 10. The lowest diversity (only three to six genera) was in semihydromorphic soils (Spruce 47, Spruce 50, Spruce 56), of which two were infected with *H. annosum s.l.* (Spruce 50, Spruce 56). This is consistent with the obtained ARDRA results.

Results of stepwise multiple regression analysis

Results of stepwise multiple regression analysis are summarized in Table 4. Total amount of soil DNA and fungal diversity estimated as the Shannon-Weaver diversity index (*H'*) were not significantly affected by any of the factors included in the regression models (not listed in Table 4). Multiple regression analysis showed that elevated concentrations of magnesium negatively affected the number of filamentous fungi and number of represented fungal genera. Elevated concentration of potassium positively affected the number of yeasts and maltose-utilizing bacteria, but negatively affected the proportion of *Mortierella* spp.

The genera *Penicillium* and *Mortierella* were chosen for regression analysis as they were abundant in all sampling plots. Forest age had significant impact on proportion of *Penicillium* spp. CFU, especially in the upper soil layer 0 to 10 cm (r = 0.63, R^2 = 0.40; Fig. 5A), and total number of fungal genera, particularly in the deepest soil layer 11 to 30 cm (r = 0.77, R^2 = 0.60; Fig. 5B). Positive impact of forest age has previously been observed on the abundance of mat-forming fungi in soil and total mat-forming taxon abundance in a mountain hemlock ecotype (Trappe et al. 2012).

Antagonism assay

Results of the antagonism assay are summarized in Appendix 1. Examples are given in Fig. 6. According to the growth in control Petri dishes, after seven days of incubation the *Penicillium* isolates were divided into three groups according to significant differences ($p < 0.05$) in growth kinetics: slow-growing with average growth rate 0.03 to 0.06 mm h^{-1}, medium fast-growing isolates with average growth rate 0.07 to 0.10 mm h^{-1} and fast-growing isolates with average growth rate 0.11 to 0.14 mm h^{-1}. The average growth rate of root rot isolates in the control plates was 0.23 to 0.30 mm h^{-1}. In particular cases the growth rate of *Penicillium* isolates changed significantly on Petri dishes in the antagonism assay (Table 5). The average growth rate of slow- and medium fast-growing isolates was significantly higher in several cases, but the average growth

Table 4. Multiple regression analysis (numbers in the columns are p values). MEA, malt extract agar. NS, not significant; –, negative impact

Factor	Yeasts and maltose-utilizing bacteria on MEA	Filamentous fungi on MEA	Percentage of *Penicillium* spp.	Percentage of *Mortierella* spp.	Total number of fungal genera
Soil sampling depth	0.0007 –	< 0.001 –	0.0007 –	NS	NS
Soil moisture content	< 0.0001	NS	0.03 –	NS	0.04 –
Soil pH_{H2O}	NS	NS	NS	NS	0.008 –
Forest age	NS	NS	0.003	NS	0.0008
Total calcium content	NS	NS	NS	NS	NS
Total magnesium content	NS	< 0.0002 –	NS	NS	0.006 –
Total potassium content	0.046	NS	NS	0.01 –	NS
C/N ratio	0.04a	NS	0.01	NS	NS
Total phosphorus content	0.05a	0.04	NS	NS	0.005
Humic acids	NS	0.004	0.04	NS	NS
Multiple R^2	0.95	0.98	0.80	0.35	0.75
p-value of the final model	< 0.0001	< 0.0001	0.0006	0.04	0.002

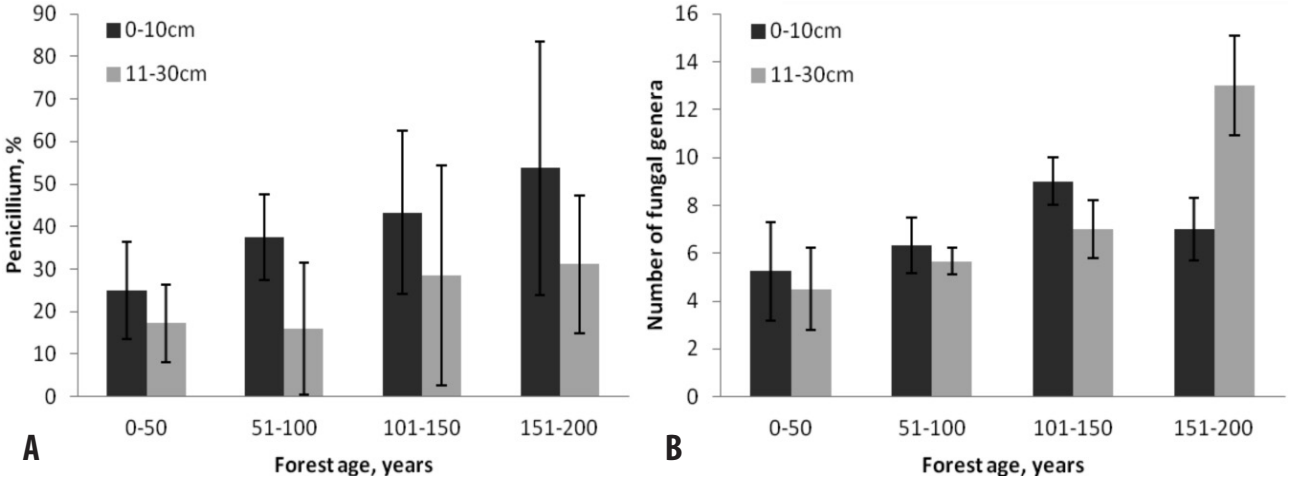

Fig. 5. Relative abundance of *Penicillium* spp. (A) and number of fungal genera depending on forest age (B).

rate of the fast-growing group was significantly reduced in antagonism pairings with all root rot isolates. The average growth rate of root rot isolates was lower by more than twofold (by 53 to 57%).

In 62% of all pairings an antagonistic zone was observed where both fungal hyphae came into contact but neither of the fungi overgrew each other. In 21% of pairings the inhibition zone after one month of incubation was still present. In the case of *P. citreonigrum,* a 1.50 to 3.75 mm wide inhibition zone was observed in pairings with all root

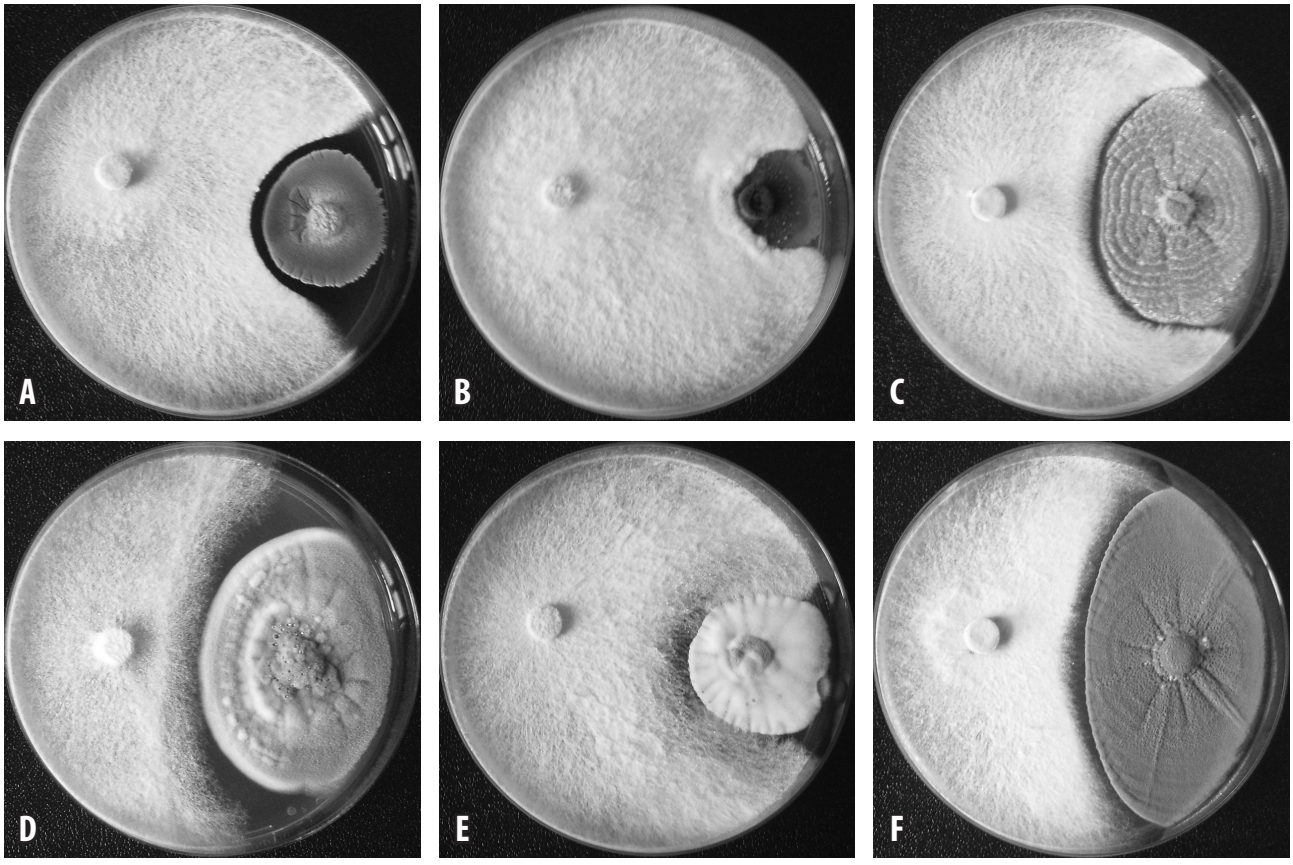

Fig. 6. Examples of antagonism assay: inhibition zone is present after one month of incubation, MSCL 981 and *P. verruculosum* MSCL 882 (A); zone of antagonism is not present and isolate of root rot overgrows *Penicillium* sp., MSCL 980 and *P. montanense* MSCL 942 (B); zone of antagonism is present but isolate of root rot partially overgrows *Penicillium* sp., S 56 and *Penicillium* sp. MSCL 1153 (C); zone of antagonism is present but fungal hyphae come into contact although none of the fungi overgrows other, MSCL 1021 and *P. canescens* MSCL 1213 (D); both fungi overgrow each other in the zone of interaction, S 56 and *P. roseopurpureum* MSCL 1220 (E); *Penicillium* sp. overgrows the isolate of root rot in the antagonism zone, S 56 and *P. thomii* MSCL 1148 (F).

Table 5. Relative changes in the average growth rate of *Penicillium* spp. isolates in the presence of different root rot isolates on Petri dishes in antagonism assay, in comparison to the control agar plates. *, significant differences among *Penicillium* groups, *p* values indicate significant changes in growth speed in comparison with control agar plates. MSCL, Microbial Strain Collection of Latvia

Group of *Penicillium*	MSCL 980	MSCL 981	MSCL 1020	MSCL 1021	S 56
Slow growing	120.69 ± 29.64 %* ($p = 0.003$)	138.32 ± 30.77 %* ($p < 0.0001$)	138.32 ± 30.77 %* ($p < 0.0003$)	102.12 ± 16.28 %	114.57 ± 12.72 % ($p = 0.0009$)
Medium fast growing	102.67 ± 17.50 %*	112.83 ± 22.43 %* ($p = 0.02$)	111.13 ± 16.92 % ($p < 0.01$)	97.09 ± 10.29 %	103.54 ± 22.25 % ($p = 0.005$)
Fast growing	71.88 ± 22.90 %* ($p = 0.001$)	76.09 ± 29.16 %* ($p = 0.008$)	73.32 ± 28.36 %* ($p < 0.01$)	80.95 ± 9.78 %* ($p = 0.0001$)	72.46 ± 16.03 %* ($p = 0.02$)

rot isolates. Only two isolates (0.8%) overgrew the isolate of root rot in the antagonism zone, *P. swiecickii* in the case of MSCL 1021 and isolate *P. thomii* in the case of S56. In 4.4% of all pairings both fungi overgrew each other in the zone of interaction. In other cases (11.8%), isolates of the root rot fungi fully or partially overgrew the *Penicillium* sp. The efficiency of *Penicillium* in suppressing radial growth of isolates of root rot fungi ranged from 26 to 84% (average 43 to 57% for aparticular isolate of root rot fungus). *Penicillium* isolates with the highest efficiency were medium- and fast-growing. *P. griseofulvum* var. *dipodomyicola* MSCL 874, isolated from the agricultural soil, had efficiency 59 to 82%, *P. brasilianum* MSCL 1229 had efficiency 46 to 69% and *P. canescens* MSCL 1213 – 54 to 69%. Both of the latter were isolated from soil of aspen stands. Efficiency of 83 to 84% in suppressing radial growth of the pathogen was observed in the case of two *Penicillium* isolates from aspen stands: *P. canescens* MSCL 1091 with *H. annosum s.s.* MSCL 1021, *Penicillium* sp. O48 with *H. annosum s.l.* S56 and one isolate from an infected spruce stand *P. spinulosum* MSCL 1145 with *H. parviporum* MSCL 980.

In general, the results obtained for isolates of the same species and *Penicillium* isolates from agricultural soil, former agricultural soil planted with aspen stands and forest soil showed similar results. *Penicillium* spp. in general reduced the growth rate of *H. annosum s.l.* and some isolates produced some antifungal components, as described previously (Yang et al. 2008) but the antagonistic effect was not as strong as in the case of *Trichoderma* spp. (Nikolajeva et al. 2012).

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Appendix 1. List of *Penicillium* isolates used in antagonism assay, their growth rate on 3% MEA and antagonistic abilities against root rot isolates MSCL 980, MSCL 981, MSCL 1020, MSCL 1021 and S56. If the inhibition zone after one month of incubation was still present its width is indicated in millimeters. (1), soil of European aspen (*Populus tremula*) and hybrid aspen (*Populus tremuloides* × *P. tremula*) stands (Grantina-Ievina et al. 2012); (2), soil of infected spruce (*Picea abies*) stands (Grantina et al. 2012.); (3), the present study; (4), agricultural soil (Grantina et al. 2011). 0, zone of antagonism is present but fungal hyphae come into contact although none of the fungi overgrows other; 00, both fungi overgrow each other in the zone of interaction; 0/–, zone of antagonism is present but isolate of root rot partially overgrows *Penicillium* sp.; +, *Penicillium* sp. overgrows the isolate of root rot in the antagonism zone; –, zone of antagonism is not present and isolate of root rot overgrows *Penicillium* sp. MSCL, Microbial Strain Collection of Latvia

Species (reference)	MSCL strain number	Growth (mm h^{-1})	MSCL 980	MSCL 981	MSCL 1020	MSCL 1021	S 56
Slow growing isolates							
P. canescens (1)	1119	0.03 ± 0.004	00	0	0	4.0	1.5
P. canescens (1)	1209	0.05 ± 0.002	2.5	0	0	6.0	3.5
P. montanense (2)	942	0.05 ± 0.000	–	0	0	0/–	0
P. canescens (1)	1227	0.06 ± 0.002	3.0	2.0	1.5	0	2.3
P. canescens (1)	1210	0.06 ± 0.000	0	0.5	0	–	0
P. canescens (1)	1208	0.06 ± 0.000	2.0	0.5	0	0/–	0
P. canescens (1)	1214	0.06 ± 0.002	0	0	0	0	0
P. canescens (1)	1109	0.06 ± 0.000	3.7	3.0	1.5	0	2.0
P. canescens (1)	1225	0.06 ± 0.003	2.0	0	0	–	0
P. canescens (1)	1211	0.06 ± 0.002	1.5	1.0	1.5	0	4.0
P. canescens (1)	1224	0.06 ± 0.005	1.5	0	0	–	1.5
P. canescens (1)	1215	0.06 ± 0.003	0	0	0	–	0
P. citreonigrum (3)	1150	0.06 ± 0.002	2.5	1.5	3.3	3.8	3.3
P. montanense (1)	1228	0.06 ± 0.002	3.5	1.0	0	–	0
P. roseopurpureum (1)	1220	0.06 ± 0.002	0	0	0	–	00
P. roseopurpureum (1)	1218	0.06 ± 0.000	0	0	0	–	00
P. roseopurpureum (1)	1219	0.06 ± 0.002	0	0	0	–	00
Penicillium sp. (3)	1152	0.06 ± 0.002	–	0	0	0/–	0
Penicillium sp. (3)	1137	0.06 ± 0.001	–	0	0	–	0
P. verruculosum (4)	882	0.06 ± 0.008	0	1.5	0	–	0
Medium fast growing isolates							
P. canescens(4)	879	0.07 ± 0.008	2.3	0	0	0/–	2.6
P. canescens (1)	1223	0.07 ± 0.001	1.5	0	0	0	0
P. canescens (1)	1104	0.07 ± 0.000	1.5	0	0	3.3	0
P. canescens (1)	1110	0.07 ± 0.006	0	0	0	1.5	1.0
P. canescens (1)	1213	0.07 ± 0.001	0	2.5	0	0	0
P. canescens (1)	1216	0.07 ± 0.002	0	0	0	–	0.8
P. canescens (1)	1107	0.07 ± 0.003	0	0	1.5	–	0
P. canescens (1)	-	0.07 ± 0.002	0	0	0	0	0
P. corylophilum (1)	1135	0.07 ± 0.001	–	0	0	–	0
Penicillium sp. (1)	-	0.07 ± 0.000	0	0	0	2.8	0
P. spinulosum (3)	1138	0.07 ± 0.001	–	0	-	–	-
P. swiecickii (1)	1221	0.07 ± 0.000	0	0	0	0	0
P. aurantiogriseum (4)	876	0.08 ± 0.002	0	0	0	0/–	0
P. aurantiogriseum (1)	-	0.08 ± 0.002	1.5	0	0	3.5	2.0
P. canescens (1)	875	0.08 ± 0.009	0	0	0	–	0
P. canescens (1)	1212	0.08 ± 0.002	0	1.8	0	1.0	0
P. canescens (1)	1226	0.08 ± 0.001	0	1.5	0	4.0	0
P. canescens (1)	-	0.08 ± 0.002	1.5	0	0	0	0
P. swiecickii (1)	1217	0.08 ± 0.002	0	0	0	+	0
P. canescens (1)	1091	0.10 ± 0.000	0	0	0	1.0	0
P. swiecickii (1)	1222	0.10 ± 0.072	1.5	0	0	0	0
Fast growing isolates							
P. canescens (1)	1093	0.11 ± 0.004	0	0	1.5	0/–	0
P. melanoconidium (4)	871	0.11 ± 0.080	0	0	0	–	0
Penicillium sp. (3)	1153	0.11 ± 0.076	00	0	0	–	0/-
P. thomii (3)	1148	0.12 ± 0.002	00	0	0	–	+
P. brasilianum (1)	1229	0.13 ± 0.004	0	0	0	–	0

Appendix 2. *continued*

Species (reference)	MSCL strain number	Growth (mm h^{-1})	MSCL 980	MSCL 981	MSCL 1020	MSCL 1021	S 56
P. griseofulvum var. *dipodomyicola* (4)	874	0.13 ± 0.000	0	0	0	0/–	0
P. janthinellum (1)	1097	0.13 ± 0.001	0	0	0	00	0
P. canescens (1)	1095	0.14 ± 0.001	0.5	0	0	–	0
P. commune (4)	878	0.14 ± 0.002	00	0	0	–	0
P. spinulosum (3)	1145	0.14 ± 0.001	00	0	0	00	0
P. spinulosum (2)	952	0.14 ± 0.001	00	0	0	–	0

Appendix 2. List of fungal species determined in the soil of sampling plots according to morphological characters or ribosomal DNA sequencing results (indicated with the number of homologue sequence at NCBI data base). MSCL, Microbial Strain Collection of Latvia

Sampling plot; depth (cm)	Species	Homologue sequence, NCBI acc. No.	Maximum identity (%)	E value	Strain No. in MSCL
Pine 80; 0–10	*Humicola* sp.	–	–	–	–
	Lecythophora mutabilis	HM036599.1	100	0	–
	Lecythophora sp.	AY219880.1	95	0	–
	Mortierella sp.	HQ022201.1	99	0	–
	Mucor sp.	–	–	–	
	Penicillium citreonigrum	AY157489.1	96	0	1150
	Phoma herbarum	AF218792.1	98	0	–
	Trichoderma sp.	–	–	–	–
Pine 80; 11–30	*Aspergillus cervinus*	–	–	–	–
	Cladosporium sp.	–	–	–	–
	Mortierella sp.	FJ810149.1	100	0	–
	Mortierella sp.	FJ810149.1	100	0	–
	Penicillium sp.	–	–	–	–
	Sagenomella sp.	GQ169325.1	98	0	–
	Trichoderma sp.	–	–	–	–
Spruce 37; 0–10	*Beauveria bassiana*	GU566276.1	99	0	1055
	Hypocrea pachybasioides	GU934589.1	99	0	1072
	Mortierella sp.	HQ022201.1	99	0	–
	Mucor sp.	–	–	–	–
	Penicillium sp.	–	–	–	–
	Pochonia bulbillosa	AB378552.1	99	0	1056
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 37; 11–30	*Hypocrea pachybasioides*	AY240841.1	98	0	–
	Mortierella sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Stilbella sp.	HQ631053.1	99	0	1057
	Penicillium glaucoalbidum	FJ903357.1	95	0	–
	Umbelopsis sp.	–	–	–	–
Spruce 47; 0–10	*Mucor* sp.	–	–	–	–
	Penicillium sp.	–	–	–	–
	Trichoderma asperellum	FJ908743.1	98	0	–
	Umbelopsis sp.	–	–	–	–
Spruce 47; 11–30	*Hypocrea viridescens*	GU566274.1	99	0	–
	Penicillium janthinellum	GU212865.1	98	0	1137
	Penicillium thomii	GU934556.1	100	0	1148
	Umbelopsis sp.	–	–	–	–

Appendix 2. *continued*

Sampling plot; depth (cm)	Species	Homologue sequence, NCBI acc. No.	Maximum identity (%)	E value	Strain No. in MSCL
Spruce 28; 0–10	*Mortierella macrocystis*	AJ878782.1	96	0	–
	Mucor sp.	–	–	–	–
	Paecilomyces carneus	AB258369.1	100	0	1132
	Penicillium spinulosum	GU566252.1	100	0	1145
	Tolypocladium cylindrosporum	AB208110.1	97	0	1129
	Trichoderma viride	FJ872073.1	99	0	–
	Umbelopsis sp.	–	–	–	–
Spruce 28; 11–30	*Ambomucor seriatoinflatus*	AY743664.1	80	1,00E–61	1142
	Mortierella macrocystis	AJ878782.1	96	0	–
	Penicillium sp.	–	–	–	–
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
	Zalerion varium	AJ608987.1	100	0	1102
Spruce 62; 0–10	*Absidia psychrophilia*	AY944874.1	93	0	1139
	Lophiostoma cynaroidis	EU552138.1	90	5,00E–145	–
	Mortierella macrocystis	AJ878782.1	99	0	–
	Mortierella macrocystis	AJ878782.1	96	0	–
	Penicillium sp.	–	–	–	–
	Pochonia bulbillosa	AB378551.1	100	0	–
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
	Zygomycete	AM292200.1	96	0	–
Spruce 62; 11–30	*Clonostachys candelabrum*	AF210668.1	98	0	1054
	Hypocrea pachybasioides	GU934589.1	99	0	–
	Lecythophora mutabilis	HM036599.1	99	0	–
	Mortierella sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 141; 0–10	*Beauveria bassiana*	AJ560684.1	98	0	1094
	Botrytis sp.	–	–	–	–
	Mortierella sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Oidiodendron sp.	HM208724.1	98	0	1125
	Penicillium sp.	–	–	–	–
	Stilbella byssiseda	AF335453.1	93	0	–
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 141; 11–30	*Beauveria bassiana*	AY532056.1	99	0	1100
	Mortierella sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Penicillium sp.	–	–	–	–
	Pochonia bulbillosa	AB378554.1	95	0	–
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 160; 0–10	*Chalara longipes*	FR717230.1	98	0	–
	Hypocrea pachybasioides	AY240841.1	100	0	1115
	Mortierella sp.	EU240133.1	99	0	–
	Mucor sp.	–	–	–	–
	Penicillium sp.	–	–	–	1153
	Thanatephorus cucumeris	EF155506.1	99	0	–
	Umbelopsis sp.	–	–	–	–

Appendix 2. *continued*

Sampling plot; depth (cm)	Species	Homologue sequence, NCBI acc. No.	Maximum identity (%)	E value	Strain No. in MSCL
Spruce 160; 11–30	*Absidia psychrophilia*	AY944874.1	94	0	–
	Ambomucor seriatoinflatus	AY743664.1	99	0	1092
	Aspergillus cervinus	–	–	–	–
	Botrytis sp.	–	–	–	–
	Cladosporium sp.	–	–	–	–
	Humicola sp.	–	–	–	–
	Mortierella macrocystis	AJ878782.1	99	0	1136
	Mortierella sp.	FJ810149.1	99	0	1053
	Mortierella sp.	FJ810149.1	99	0	–
	Penicillium canescens	FJ439586.1	99	0	–
	Penicillium spinulosum	GU566247.1	98	0	1138
	Stilbella sp.	HQ631053.1	99	0	1144
	Tolypocladium cylindrosporum	AB208110.1	97	0	–
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
	Verticillium sp.	–	–	–	–
Spruce 50; 0–10	*Penicillium* sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 50; 11–30	*Penicillium* sp.	–	–	–	–
	Mucor sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 56; 0–10	*Mortierella* sp.	–	–	–	–
	Penicillium sp.	GU446648.1	97	0	1152
	Talaromyces sp.	GU827480.1	99	0	1147
	Trichoderma sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–
Spruce 56; 11–30	*Mortierella* sp.	–	–	–	–
	Hypocrea citrina	DQ000622.1	99	0	–
	Lecythophora mutabilis	HM036599.1	99	0	–
	Penicillium sp.	–	–	–	–
	Umbelopsis sp.	–	–	–	–