Environmental and Experimental Biology (2011) 9: 9–21 *Original Paper*

The influence of the land use on abundance and diversity of soil fungi: comparison of conventional and molecular methods of analysis

Lelde Grantina[1]*, Elina Seile[1], Kristine Kenigsvalde[1], Raimonds Kasparinskis[2], Guntis Tabors[3], Vizma Nikolajeva[1], Pieter Jungerius[4], Indrikis Muiznieks[1]

[1]Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, Riga LV-1586, Latvia
[2]Department of Environmental Science, Faculty of Geography and Earth Sciences, University of Latvia, Alberta 10, Riga LV-1010, Latvia
[3]Department of Botany and Ecology, Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, Riga LV-1586, Latvia
[4]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

*Corresponding author, E-mail: lelde.grantina@lu.lv

Abstract

This study examined fungal communities in 11 soil profiles. The objective was to apply conventional and molecular microbiology methods to obtain baseline information on the general characteristics of soil microbial communities in relation to land use – forest, former agricultural land, meadow and arable land. The number of filamentous fungi species and the total number of cultivable microorganisms showed a tendency to decrease with increasing depth. The diversity of fungi obtained with amplified rDNA gene restriction analysis was similar for all studied land use groups, diversity decreased with soil depth, especially in soils of former agricultural land and meadow. The proportion of fungal DNA as part of the total soil DNA was significantly higher in forest and abandoned land soil than in agricultural soil. The amount of *Trichoderma* spp. DNA was similar for all land groups, but its relative amount as percentage of total fungal DNA was higher in meadow and arable land soil. The land use type had a significant impact only on the diversity of cultivable soil fungi and fungal DNA amount. Soil depth and moisture content had a greater effects.

Key words: ARDRA, fungal diversity, Shannon-Weaver diversity index, soil, quantitative PCR, *Trichoderma*.
Abbreviations: AME, agarised malt extract; ARDRA, amplified ribosomal DNA gene restriction analysis; CFF, cultivable filamentous fungi; qPCR, quantitative PCR; CFU, colony forming units.

Introduction

Soil is a heterogeneous environment and different components of the solid fractions in soil (sand, silt, clay, organic matter etc.) provide variable microhabitats. Major interrelated factors affecting microbial diversity in soil include soil forming processes, physicochemical properties of soil, soil particle size distribution, vegetation and land use type (management). The relative effects of these factors differ in different soil types, horizons and climatic zones (Garbeva at al. 2004). Several studies investigating the impact of different plant cultures and soil types have shown, that the soil type has the greatest influence on the structure of microbial populations (Chiarini et al. 1998; Grayston et al. 1998; Buyer et al. 1999; Marschner et al. 2001; Wieland et al. 2001), although under some conditions the influence of the vegetation may prevail, e.g. the maize rhizosphere accommodates specific bacterial communities, the structures of which change with the developmental state of the plant (Baudoin et al. 2002). Many publications emphasize the influence of the land use (agricultural land, grassland, tropical forest and pasture, etc.) on the structure of soil microbial communities (Nusslein, Tiedje 1999; McCaig et al. 2001; Webster et al. 2002; Clegg et al. 2003; Drijber et al. 2003).

Abiotic conditions of soil (pH, moisture content and temperature) affect soil microorganisms in all soil land use types (Jurgensen et al. 1997; Hackl et al. 2004; Setälä, Mc Lean 2004; Borken et al. 2006; Ruisi et al. 2007; Zachow et al. 2009). It is known that fungi in soil constitute a significant part of the soil biomass and they have several important functions in soil, such as decomposition of organic material, nutrient cycling, formation of soil aggregates and mycorrhizal symbiosis (Kabir et al. 2003). Most (more than 80 %) soil fungi and bacterial species are not cultivable under laboratory conditions (Leckie 2005).

Two main approaches can be employed to study soil microbial communities – conventional plating of cultivable microorganisms and molecular methods that are independent of cultivation. Both approaches are associated

with specific advantages and disadvantages. Only a small portion of all microorganisms in soil can be cultivated in standard laboratory conditions, and the isolation of pure cultures and lengthy effort are needed for the determination of taxonomic identity of the isolates. In contrast, molecular methods provide general insight into the genetic heterogeneity of soil microbial communities and allow to identify specific microorganisms without isolation, but they are highly dependent on the success of the isolation of DNA from soil, eventual presence of the DNA amplification or restriction inhibitors, choice of the primers, discriminating power of analysis etc. (Kowalchuk et al. 2006).

Amplified ribosomal DNA gene restriction analysis (ARDRA) can be used for genetic fingerprinting of simple commu-nities, populations or phylogenetic groups. High resolution discrimination can be obtained at the species level. In soil microbiology this method is used to determine diversity within phylogenetic or functional groups of microorganisms (Lynch et al. 2004). Digested PCR products can be run in polyacrylamide (Perez-de-Mora 2006) or in agarose gel (Smit et al. 1997; Schwieger, Tebbe 2000; Chabrerie et al. 2003). Usually at least two restriction enzymes are used and the obtained fragments can be analyzed as separate (Schwieger, Tebbe 2000; Klamer, Hedlund 2004) or combined (Wang et al. 2008) data sets.

The abundance of the specific groups of microorganisms in soil can be determined by quantitative PCR (qPCR) (Filion et al. 2003; Kabir et al. 2003; Kolb et al. 2003; Smits et al. 2004; Fierer et al. 2005). This method has been employed to quantify amounts of the genus *Trichoderma* fungi in soil by estimating their potential to counteract the activity of fungal plant pathogens (Cordier et al. 2006).

The objective of our investigation was to apply conventional and molecular microbiology methods in order to obtain baseline information about general characteristics of Northern temperate zone soil microbial communities depending on land use – forest, abandoned (former) agricultural land, meadow and arable land. We characterized and compared soil microbial communities employing conventional plating and molecular methods, ARDRA and qPCR. The number of cultivable microorganisms (colony forming units, CFU) was determined using the plate count method. Abundance of representatives of the typical fungal genera was determined by light microscopy of colonies. A robust culture-independent ARDRA and qPCR with universal fungal primers and *Trichoderma* spp. specific primers were used as molecular methods. The Shannon-Weaver diversity index (*H'*) was determined for the communities.

Materials and methods

Sampling plots, soil sampling and estimation of soil pH

Samples from soil profiles or outcrops were taken with an auger from the Ap, A1 and B horizons of agricultural soils and O, Ah and B horizons of forest soils (0 – 10 cm, 10 – 30 cm and 30 – 40 cm deep), on August 2007 in Jelgava district (Latvia), and on October 2007 in the Cesis and Valka districts (Latvia). The sampling sites were divided in the four groups: forest land; former agricultural land; agricultural land – meadow; agricultural land – conventional arable land. The characteristics of the soil profiles are given in Table 1. Samples were placed in sterile plastic bags (*Nasco* WHIRL-PAK) and stored at 4 °C for a few days until plating of cultivable microorganisms and later stored at –20 °C. The pH of the soil samples was measured in distilled water (ISO 10390). The moisture content of the soil was determined according to ISO 11465.

Extraction and quality control of total soil DNA

Total soil DNA was extracted from the collected samples by harsh lysis using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc.). Samples (250 mg) were homogenized using a horizontal Mixer Mill Type MM 301 (Retsch) at 1800 oscillations min^{-1} for 10 min. DNA from each sample was extracted twice. The amount and purity of the DNA was assessed by spectrophotometry (Ultrospec 3100 Pro; Amersham Biosciences) and by 1% agarose gel electrophoresis.

PCR and ARDRA of soil DNA

The fungal ribosomal RNA gene region containing two internal transcribed spacers and the 5.8S rRNA gene (ITS1-5.8S-ITS2) was amplified with primers ITS1F and ITS4 (Gardes, Bruns 1993). These primers amplify the ITS1-5.8S-ITS2 region of *Ascomycota*, *Basidiomycota* and *Zygomycota* fungi. Soil samples were analyzed undiluted, or in presence of some inhibitors in 1:10 and 1:100 dilutions.

In a Eppendorf Personal Mastercycler the reactions were carried out in 50 µL with 2 u of Hot Start *Taq* DNA Polymerase in 1× Hot Start PCR Buffer containing 0.2 mM each NTP, 8 mM $MgCl_2$ (Fermentas), 0.5 µM each primer (OPERON Biotechnologies) and 1 µl of DNA template. The PCR conditions were: 4 min at 95 °C, (40 s, 95°C; 40 s, 52 °C, 60 s, 72 °C) × 30 cycles and 10 min at 72 °C.

The PCR amplification products were precipitated by 450 µL of 96% ethanol and 3 M sodium acetate, pH 5.0 (19:1). After 15 min incubation at –20 °C, samples were centrifuged in a Sigma 1-15P centrifuge for 15 min at 14 000 rpm in room temperature, washed with 70% ethanol, dried and dissolved in 16 µL of sterile distilled water. The DNA was divided in two parts and digested with restriction endonucleases *Bsu*RI (Chabrerie et al. 2003) and *Eco*RI (Fermentas) separately. Restriction products were run in 2% agarose gels, photographed with a BioSpectrum AC Imaging System and analyzed with software KODAK1D. For the estimation of the Shannon-Weaver diversity index (H'_{ARDRA}) the following equation was used:

$H'_{ARDRA} = -\Sigma\, p_j \log_2 p_j$, where p_j is a relative intensity of individual band (Gabor et al. 2003).

Table 1. Soil profiles and their characterization. [1]The average pH (± SD) calculated from the pH measurements of all analyzed depths. [2]Sampling date 26.10.2007, average air temperature 4.02 °C. [3]Forest type according to the classification of Buss (1997). [4]Sampling date 27.10.2007, average air temperature 5.23 °C. [5]Sampling date 07.08.2007, average air temperature 20.61 °C. [6]Sampling date 08.08.2007, average air temperature 22.74 °C

Location	Soil profile	Soil type		Vegetation	Number of soil samples	pH	Soil moisture content at each depth (%)
Forest lands							
Cesis district, Taurene[2]	Forest1	Typical podzol	Haplic Cambisols	*Myrtillosa* forest type – spruce (*Picea abies)*, pine (*Pinus sylvestris*), birch (*Betula pendula*), gray alder (*Alnus incana*)	3	5.12 ± 0.23	42.5; 8.4; 8.0
Valka district, Strenči[4]	Forest2	Typical podzol	Haplic Cambisols	*Vacciniosa* forest type – spruce (*Picea abies*), pine (*Pinus sylvestris*), birch (*Betula pendula*)	3	5.30 ± 0.53	6.6; 3.0; 8.2
Valka district, Strenči[4]	Forest3	Typical podzol	Haplic Arenosols	*Cladinoso-callunosa* forest type – pine (*Pinus sylvestris*) monoculture	3	4.46 ± 0.62	4.5; 2.9; 45.0
Former agricultural lands							
Cesis district, Taurene[2]	Former1	Eroded soil	Haplic Luvisols	Natural afforestation with spruce (*Picea abies*)	3	7.53 ± 0.20	15.7; 19.3; 21.4
Cesis district, Taurene[2]	Former2	Colluvial soil	Haplic Luvisols	Natural afforestation with spruce (*Picea abies*)	3	6.68 ± 0.29	18.1; 17.6; 22.6
Agricultural lands, meadows							
Jelgava district, Svete[5]	Meadow1	Gleyic sod-podzolic soil	Stagnic Cambisols	Regularly cut and pastured	3	7.90 ± 0.31	10.2; 10.6; 8.3
Jelgava district, Svete[5]	Meadow2	Granular alluvial soil	Fluvic Cambisols	Regularly cut and pastured	3	7.44 ± 0.26	14.1; 13.2; 12.9
Jelgava district, Svete[5]	Meadow3	Pseudogley sod-podzolic soil	Endogleyic Umbrisols	Regularly cut and pastured	2	7.72 ± 0.06	10.7; 10.4
Agricultural lands, conventional arable lands							
Jelgava district, Svete[6]	Field1	Pseudogley sod-podzolic soil	Endogleyic Umbrisols	Arable land	2	7.55 ± 0.07	10.4; 9.8
Jelgava district, Svete[6]	Field2	Sod-pseudogley soil	Mollic Luvic Stagnosols	Arable land	3	7.69 ± 0.11	14.2; 12.7; 10.9
Jelgava district, Svete[6]	Field3	Sod-gleyic soil	Endogleyic Umbrisols	Arable land	2	6.96 ± 0.06	10.6; 13.2

Analysis of cultivable microorganisms

In addition to the utilized molecular methods, cultivable microorganisms in three replicates were obtained from soil samples from forest and former agricultural land to estimate the CFU of filamentous fungi, bacteria and yeasts. Soil sample serial dilutions were prepared (Pepper et al. 1995; Alef, Nannipieri 1998). Agarised malt extract (AME) (30 g L^{-1}, pH 5.4 ± 0.2, Biolife) was used as a growth medium, as it supports growth of filamentous fungi, yeasts and some groups of bacteria. In our work, the total number of cultivable microorganisms was estimated as the number of CFU of the microorganisms per gram of dry soil on AME after 120 h at 20 ± 2 °C (Vanderzant, Splittstoesser 1992).

Genera of cultivable filamentous fungi (CFF) were identified using keys (Barnett 1957; Kiffer, Morelet 2000) and light microscopy of the morphology of pure cultures.

For the estimation of the Shannon-Weaver diversity index of CFF (H'_{CFF}) the following equation was used:

$H'_{CFF} = -\Sigma\, p_j \log_2 p_j$, where p_j is a relative abundance of particular genera of CFF.

Quantitative PCR

In addition to the methods described in previous chapters, total soil DNA from the upper horizons was subjected to qPCR. In qPCR either the ITS1-5.8S-ITS2 rDNA region of higher fungi with primers ITS1F and ITS4 or of *Trichoderma* spp. with the primers uTr and uTf (Hagn et al. 2007) was used to determine the amount of total fungal DNA and *Trichoderma* spp. DNA as part of the total soil DNA. Each soil sample was analyzed in three replicates.

In a SmartCycler (Cepheid) the reactions were carried out in 25 µL containing 12.5 µL SYBR® Premix Ex Taq (TaKaRa), 1 µM of each primer and 1 µL of the DNA template. The PCR conditions were: 30 s at 95 °C, (30 s, 95 °C; 30 s, 55 °C for primers ITS1F and ITS4 or 60 °C for primers uTr and uTf; 60 s, 72 °C) × 35 cycles for primers ITS1F and ITS4 or × 40 cycles for primers uTr and uTf. To quantify fungal DNA, qPCR using serial dilutions of DNA from pure cultures of *Heterobasidion parviporum*, *Penicillium lanoso-viride* MSCL 1 and *Trichoderma harzianum* MSCL 309 were performed and standard graphs were built (Fig. 1A). The average values of all three cultures were used for the calculation of the calibration curve for the total amount of the fungal DNA in soil. A standard graph (Fig. 1B) using serial dilutions of DNA of *T. harzianum* was used for the calculation of the concentration of trichodermal DNA in soil. In order to estimate the total copy number of the fungal DNA we assumed that the average fungal genome size was 35 Mb (Gregory et al. 2007) and that the average trichodermal genome size was 36.5 Mb (Kullman et al. 2005).

Statistical analysis

In order to characterize the influence of human activity upon soil microbial communities we classified the soil types in four land use groups according to the increasing intensity of human impact: 1, forest land; 2, former agricultural land; 3, meadow; 4, agricultural land. The F-test, t-test ($\alpha = 0.05$) and correlation analysis were conducted using MS *Excel*, and the program R was used for multiple regression analysis.

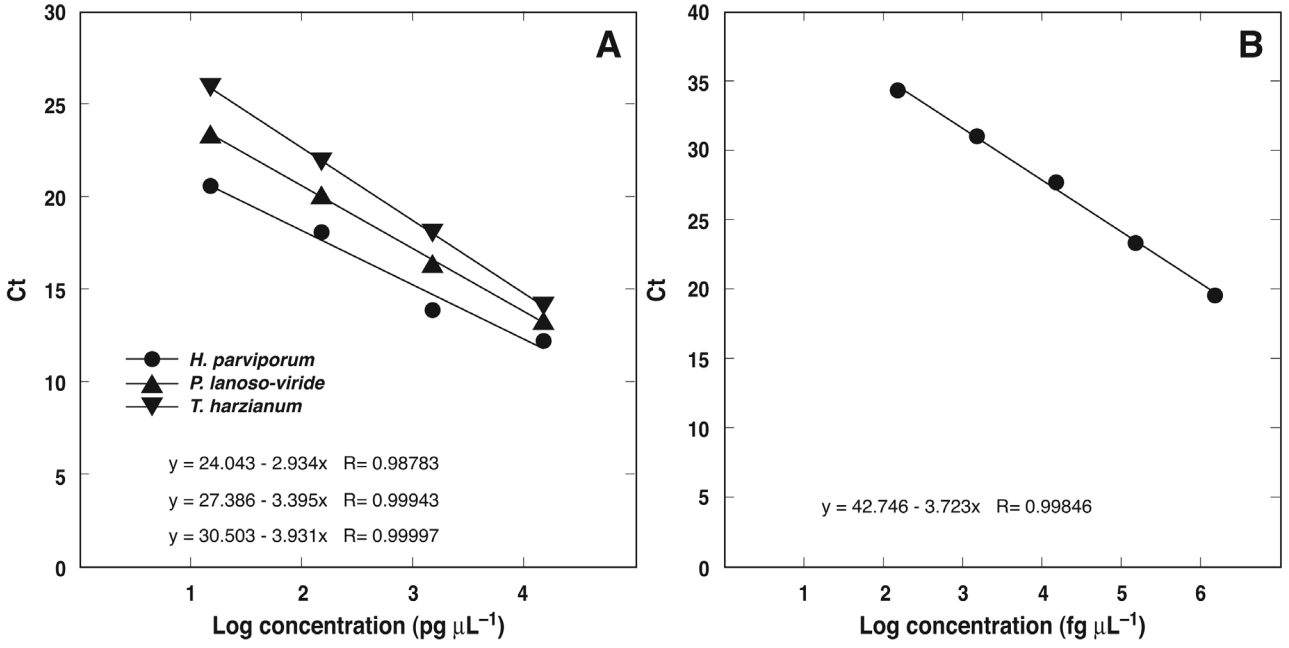

Fig. 1. Standard graphs for qPCR with *H. parviporum, P. lanoso-viride* and *T. harzianum* DNA dilution series with universal fungal primers (A) and standard graph with *Trichoderma harzianum* DNA dilution series with primers uTr and uTf (B).

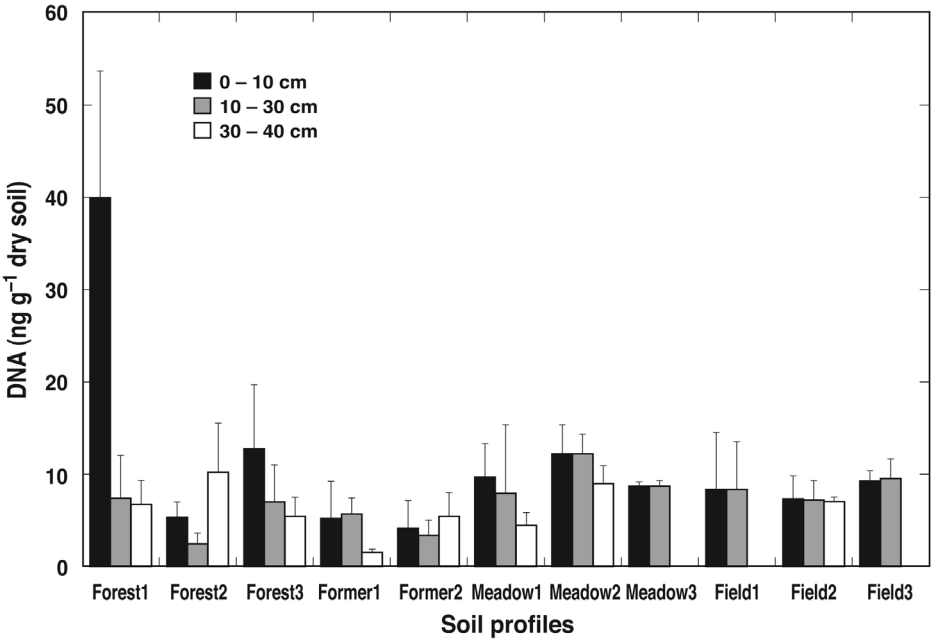

Fig. 2. Total soil DNA amount (mean ± SD, n = 2). Total soil DNA was extracted from 0.25 g of soil using PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc.).

Results

The amount of total soil DNA

The amount of total soil DNA ranged from 1.53 in deeper soil layers of former agricultural lands up to 30.28 μg g^{-1} of dry soil in upper horizons of forest soils (Fig. 2). The A_{260}/A_{280} ratio ranged from 1.6 to 1.8. In agarose gel electrophoresis the isolated DNA migrated as a diffuse band of a high molecular weight (>11 kb) region (data not shown).

Results of ARDRA

Typical ARDRA gels are shown in Fig. 3. We combined H'_{ARDRA} results from both restriction reactions (Fig. 4) as suggested by Wang et al. (2008).

Only some soil profiles (Forest1, Former2, Meadow1, Meadow3 and Field2) showed significant differences (t-test) in H'_{ARDRA} values between samples from different soil depths. In all soil profiles, the highest diversity of fungi was observed in the upper soil layer, with the exception of profiles Forest2 and Field2. In those two profiles the highest H'_{ARDRA} was observed at a depth of 10 – 30 cm. In profile Forest3 the fungal diversity was significantly higher ($p = 0.003$) than in the other two forest profiles.

In all the land use types, the mean fungal diversity H'_{ARDRA} was similar foe any of the examined sampling depths. In the 0 – 10 cm depth the H'_{ARDRA} varied from 2.61 ± 0.56 till 3.04 ± 0.24. At depth 10 – 30 cm, a significantly ($p = 0.002$) lower diversity occurred in meadows compared to other land use types – 2.26 ± 0.08 vs. 2.73 ± 0.29. In the deepest layer the H'_{ARDRA} varied from 1.95 ± 0.57 to 2.48 ± 0.48.

The number of cultivable microorganisms, dominant fungal genera and diversity of CFF

The number of CFF in soil samples (Fig. 5) from forest and former agricultural soils decreased significantly ($p < 0.05$) with increasing depth of sampling seen by comparing the upper soil layer (0 – 10 cm) with deeper layers (10 – 30 cm and 30 – 40 cm). The total number of CFU of

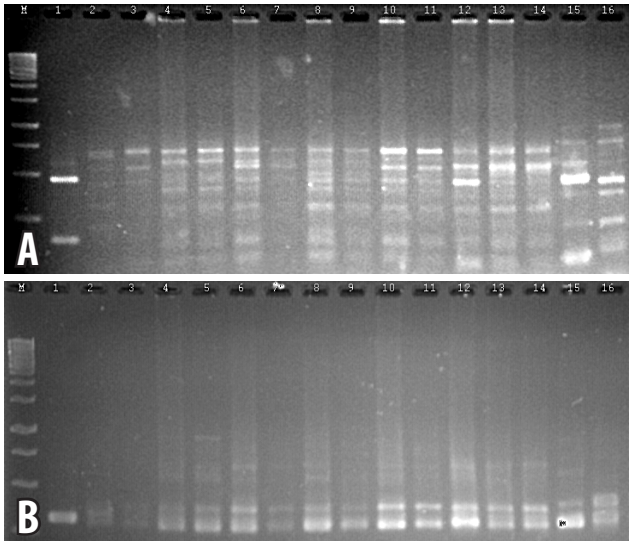

Fig. 3. Examples of ARDRA results in 2 % agarose gels. A – restriction with *Bsu*RI. B – restriction with *Eco*RI. Lanes in gels A and B: M-Gene Ruler 1 kb DNA Ladder (Fermentas). 1. Positive control with *T. harzianum* DNA. 2. Forest1, 30-40 cm. 3. Forest2, 0-10 cm. 4. Meadow2, 0-10 cm. 5. Meadow2, 10-30 cm. 6. Meadow3, 0-10 cm. 7. Meadow3, 10-30 cm. 8. Field1, 0-10 cm. 9. Field1, 10-30 cm. 10. Field2, 0-10 cm. 11. Field2, 10-30 cm. 12., 13. Field2, 30-40 cm. 14. Field2, 0-10 cm. 15., 16. Field2, 30-40 cm.

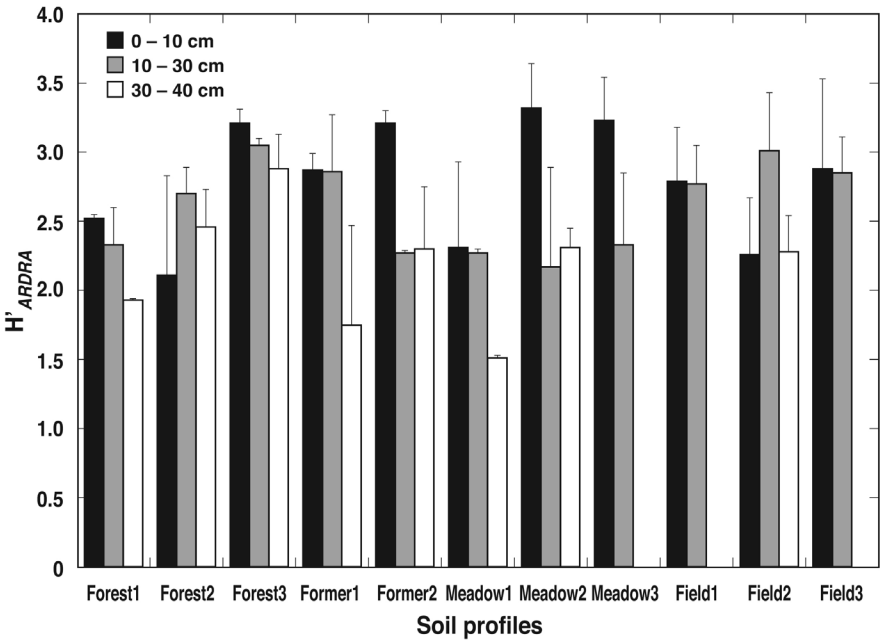

Fig. 4. Average Shannon-Weaver diversity index H'_{ARDRA} of fungal diversity in all analyzed land use groups (mean ± SD, n = 2).

microorganisms also decreased with increasing depth of sampling in soil profiles Forest1, Forest3 and Former1 (Fig. 6), but not in profiles Forest2 and Former2. In profile Forest1 the differences were statistically significant ($p < 0.05$) for all of the soil levels. In contrast to the other soils, profile Forest2 had a significantly ($p < 0.05$) increased number of cultivable microorganisms at a depth of 30 – 40 cm in comparison to the depth 0 – 30 cm. In soil profile Former2 the total number of cultivable microorganisms was similar in all horizons.

In general the amount of CFF was higher (statistically not significantly) in forest land soil in comparison with former agricultural lands, but the total count of CFU of microorganisms was higher (not significantly) in former agricultural land soil (Fig. 5, Fig. 6).

The most abundant genera and groups of fungi in soils are listed in Table 2. The identified fungi belonged either to *Ascomycota* or *Zygomycota*. Sterile mycelia and also *Penicillium* and *Mucor* were in all samples. Sterile mycelia were abundant in all of the analyzed profiles. In deeper soil layers of Forest1, Forest2 and Former2 profiles, sterile mycelia made up more than 90% of CFF.

H'_{CFF} index tended to be higher (not significantly) in forest soils than in former agricultural soils (Fig. 7).

Results of quantitative PCR

The highest amount of fungal DNA was found in forest soils, and the lowest in arable lands (no significant difference). The proportion of fungal DNA of the total soil DNA was the highest in the forest lands and former agricultural lands and lowest in meadows and fields (differences between these two groups was significant, $p = 0.02$). The amount of *Trichoderma* spp. DNA was similar in all the land use types, but the proportion of *Trichoderma* spp. DNA was higher in meadows and arable lands, in comparison to the other land use types, $p = 0.0006$ (Table 3).

Results of statistical analysis

According to multiple regression analysis (Table 4), sampling depth significantly affected all analyzed parameters; the number of CFF, total number of microorganisms, H'_{CFF}, H'_{ARDRA} and total soil DNA amount significantly decreased with depth. The strongest correlation was observed for number of CFF (Pearson correlation coefficient $r = -0.73$ to -0.83) and H'_{CFF} ($r = -0.54$ to -0.75).

In multiple regression analysis, soil moisture content had a significant positive effect on number of CFF ($p = 0.01$; $r = 0.23$). Similarly, the total soil DNA amount was positively affected by soil moisture ($p = 0.003$; $r = 0.42$). The fungal DNA amount and *Trichoderma* spp. DNA amount were positively affected by soil moisture ($p < 0.001$; $r = 0.71$ and $p = 0.01$; $r = 0.69$, respectively).

The mean air temperature on the sampling day (see Table 1) was significantly correlated with number of CFF ($p = 0.006$, $r = 0.35$) and fungal DNA proportion of total soil DNA amount ($p = 0.03$, $r = -0.65$) but not with total soil DNA ($p = 0.02$; no correlation). A strong negative correlation was observed with fungal DNA amount ($r = -0.81$) and with *Trichoderma* spp. DNA percentage of fungal DNA amount ($r = 0.85$) but this was not confirmed in multiple regression analysis.

Land use intensity had a negative impact on H'_{CFF} ($p = 0.004$, $r = -0.61$), total soil DNA amount ($p = 0.02$; $r = 0.24$), fungal DNA amount ($p < 0.001$; $r = -0.85$), and a positive impact on the proportion of *Trichoderma* spp. DNA ($p < 0.001$, $r = 0.90$).

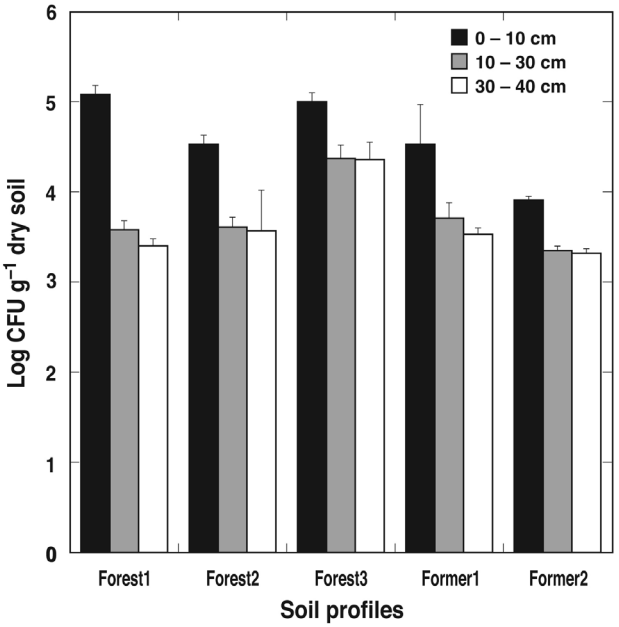

Fig. 5. The number of CFF in soil profiles in forests and former agricultural lands (mean ± SD, n = 3). The number of CFF was estimated on MEA after five days of incubation at 20 ± 2 °C.

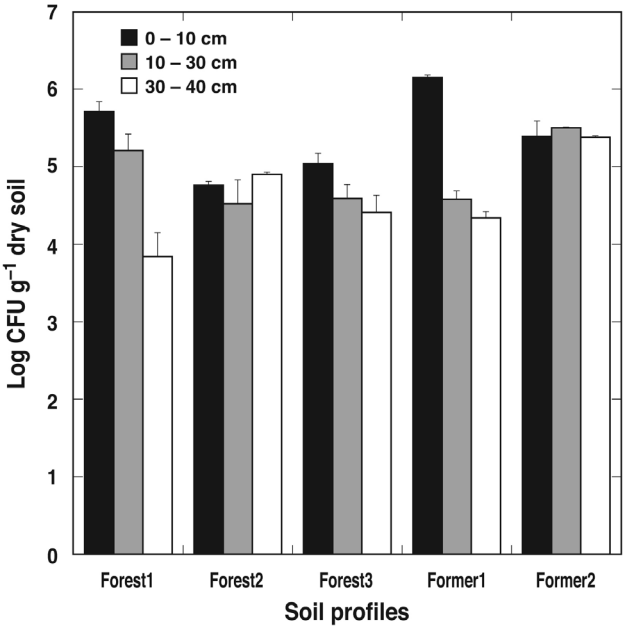

Fig. 6. Total number of cultivable microorganisms of soil profiles in forests and former agricultural lands (mean ± SD, n = 3). The number of cultivable microorganisms was estimated on MEA after five days of incubation at 20 ± 2 °C.

Table 2. Predominant fungal genera and groups

Fungi	Forest1	Forest2	Forest3	Former1	Former2
Ascomycota					
Penicillium	✓	✓	✓	✓	✓
Aspergillus	✓	✓	✓	✓	
Trichoderma	✓	✓	✓		
Verticillium			✓		
Acremonium					✓
Eladia saccula			✓		
Fusarium				✓	
Geomyces			✓		
Paecilomyces					✓
Spicaria					✓
Zygomycota					
Mucor	✓	✓	✓	✓	✓
Mortierella	✓				
Sterile mycelia	✓	✓	✓	✓	✓

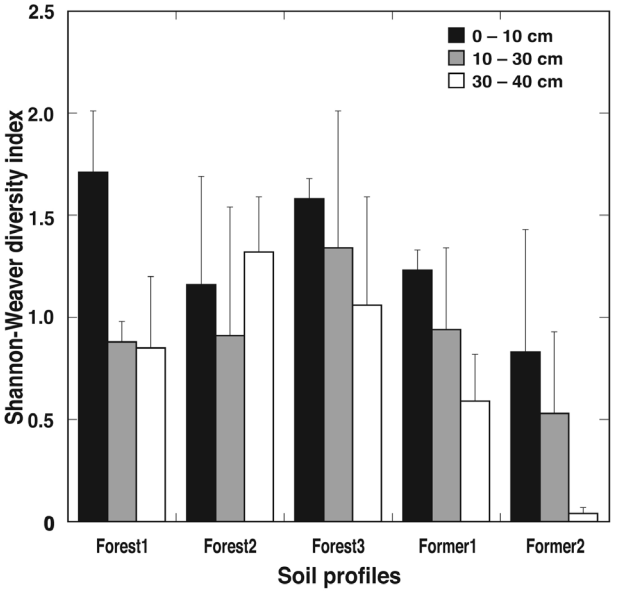

Fig. 7. Shannon-Weaver diversity index H'_{CFF} of soil profiles in forests and former agricultural lands (mean ± SD, n = 3). H'_{CFF} was calculated using CFU number of each CFF genus estimated on MEA after 10 days of incubation at 20 ± 2 °C.

Multiple regression analysis showed that soil pH affected significantly only the number of CFF (p = 0.01; r = –0.40), but correlation analysis showed a positive correlation also with H'_{CFF} (r = –0.53), and fungal DNA amount (r = –0.68), and negative correlation with proportion of affected fungal DNA (r = –0.57).

Discussion

Comparison of conventional and molecular methods

The correlation between the fungal diversity indexes obtained by conventional plating methods (H'_{CFF}) and by molecular methods (H'_{ARDRA}) was weak (r = 0.45). A stronger correlation of H'_{ARDRA} was found with number of CFF (r = 0.50 – 0.61), total soil DNA amount (r = 0.54 – 0.72), and fungal DNA amount (r = 0.58). Values of H'_{ARDRA} were always higher than those of H'_{CFF}. The diversity estimated by conventional methods used data only on filamentous fungi

Table 3. The amount of fungal and *Trichoderma* spp. DNA in the upper soil layer (0 – 10 cm), n = 3

Profile	Total soil DNA (μg g^{-1} dry soil ± SD)	Fungal DNA [ng g^{-1} dry soil ± SD (% of total DNA)]	Number of rDNA copies g^{-1} dry soil	*Trichoderma* spp. DNA [ng g^{-1} dry soil ± SD (% of fungal DNA)]	Number of *Trichoderma* spp. DNA copies g^{-1} dry soil
Forest1	30.28 ± 13.78	1223.01 ± 247.06 (7.03)	$(3.24\pm0.65) \times 10^7$	10.27 ± 1.62 (0.79)	$(2.61 \pm 0.41) \times 10^5$
Forest2	4.17 ± 1.67	220.80 ± 22.22 (5.66)	$(5.84\pm0.59) \times 10^6$	1.57 ± 0.19 (0.64)	$(3.99 \pm 0.48) \times 10^4$
Forest3	9.98 ± 4.01	683.82 ± 116.44 (7.20)	$(1.81\pm0.31) \times 10^7$	2.33 ± 0.48 (1.01)	$(5.91 \pm 1.22) \times 10^4$
Average	14.81	709.21 (4.17)	1.88×10^7	4.72 (0.81)	1.20×10^5
Former1	8.09 ± 4.03	254.70 ± 39.15 (3.15)	$(6.74\pm1.04) \times 10^6$	2.14 ± 0.66 (0.84)	$(5.43 \pm 1.68) \times 10^4$
Former2	7.69 ± 5.00	260.84 ± 30.60 (3.39)	$(6.90\pm0.81) \times 10^6$	5.49 ± 0.33 (2.11)	$(1.39 \pm 0.08) \times 10^5$
Average	7.89	257.77 (3.27)	6.82×10^6	3.82 (1.48)	9.70×10^4
Meadow1	9.66 ± 3.61	136.00 ± 2.12 (1.41)	$(3.60\pm0.06) \times 10^6$	5.14 ± 0.26 (3.78)	$(1.30 \pm 0.07) \times 10^5$
Meadow2	12.18 ± 3.12	192.93 ± 7.46 (1.58)	$(5.11\pm0.20) \times 10^6$	5.55 ± 0.21 (2.87)	$(1.41 \pm 0.05) \times 10^5$
Meadow3	8.40 ± 6.18	106.68 ± 5.03 (1.27)	$(2.82\pm0.13) \times 10^6$	3.13 ± 0.63 (2.93)	$(7.94 \pm 1.60) \times 10^4$
Average	10.08	145.02 (1.42)	3.84×10^6	4.61 (3.19)	1.17×10^5
Field1	7.06 ± 0.47	103.44 ± 9.87 (1.47)	$(2.74\pm0.26) \times 10^6$	5.16 ± 0.40 (4.99)	$(1.31 \pm 0.10) \times 10^5$
Field2	5.27 ± 2.48	113.84 ± 23.83 (2.16)	$(3.01\pm0.63) \times 10^6$	3.05 ± 1.03 (2.68)	$(7.74 \pm 2.61) \times 10^4$
Field3	3.47 ± 4.28	84.47 ± 13.42 (2.43)	$(2.24\pm0.36) \times 10^6$	3.81 ± 0.16 (4.51)	$(9.67 \pm 0.41) \times 10^4$
Average	5.27	100.58 (2.02)	2.66×10^6	4.01 (4.06)	1.02×10^5

Table 4. Impact of different factors on the analyzed parameters. NS, not significant; NE, not estimated

	Number of CFF	Total number of microorganisms	H'_{CFF}	H'_{ARDRA}	Fungal DNA amount	*Trichoderma* spp. DNA amount
Sampling depth	p = 0.0001 (negative)	p = 0.01 (negative)	p = 0.007 (negative)	p = 0.005 (negative)	NE (negative)	NE (negative)
Soil moisture content	p = 0.01	NS	NS	NS	p < 0.001	p = 0.01
Soil pH	p=0.01 (negative)	NS	NS	NS	NS	NS
Average air temperature of the sampling day	p = 0.006	NS	NS	NS	NS	NS
Land use type	NS	NS	p = 0.004 (negative)	NS	p < 0.001 (negative)	NS
Multiple R^2	0.81	0.49	0.66	0.28	0.97	0.59
Probability of the model	p = 0.0003	p = 0.02	p = 0.001	p = 0.01	p < 0.001	p = 0.03

and excluded yeasts. In molecular analysis the diversity of yeast genomes also contributed to the estimated diversity, since they are amplified with primers ITS4 and ITS1F alongside with other representatives of *Ascomycota* or *Basidiomycota*. Further, conventional methods can identify cultivable microorganisms, while the H'_{ARDRA} index reveals the diversity "of some of the most abundant community members" (Kowalchuk et al. 2006). In other investigations the detection threshold of ARDRA gels is quite high, and can detect the DNA of two different strains in various proportions at ratios as low as 1/20 (Grundmann, Normand 2000).

In most soil profiles the highest H'_{ARDRA} and H'_{CFF} index was found in the upper soil layers. However, in two soil profiles (Forest2 and Field2), the highest diversity H'_{ARDRA} was found at a depth of 10 – 30 cm, and in the Forest2 soil profile the richest community of H'_{CFF} was in the deepest soil layer (30 – 40 cm). These differences were probably not caused by soil moisture or soil pH, but can be explained by the history of soil profile development (see further).

In profile Forest3, the estimated fungal diversity was significantly higher than in the other two forest profiles, which might be caused by different soil types (Haplic Arenosols in Forest3 compared to Haplic Cambisols in the

other two forest profiles) and/or with the associated forest vegetation type.

In North Carolina in two forest sites with loblolly pine (*Pinus taeda*) and mixed hardwood the highest fungal richness was in the O horizon (organic material and detritus), intermediate values were obtained in horizons L (litter) and A (organic matter-rich mineral soil), and the lowest in the B horizon (clay-rich mineral soil) (O'Brien et al. 2005). The authors found that in general the diversity and richness was correlated positively with clone library size.

The highest diversity of cultivable microfungi and yeasts in Austria was found in the top layer of the forest soil (0 – 15 cm) without temporal flooding (Wuczkowski et al. 2003). This layer of the soil profile was characterized as a zone of the highest degradation of the organic material. These results are similar to ours with the exception of the soil profile Forest2. In agricultural soils with conventional farming the highest diversity was recorded at a depth of 30 – 35 cm caused by agricultural practices (Wuczkowski et al. 2003). In our investigation the H'_{ARDRA} diversity in agricultural soil at a depth of 10 – 30 cm was similar or slightly higher than that in the upper soil layer. In the case of the profile Field2, the diversity was lower in deeper soil (depth of 30 – 40 cm). Since this soil layer was not analyzed in the other two fields, it is difficult to make any general conclusions about the fungal diversity in this depth.

In soils of Sweden, the fungal species richness investigated using terminal-restriction fragment length polymorphism was significantly higher in newly abandoned agricultural land than in actively used agricultural fields and forest (Klamer, Hedlund 2004). In our investigation former agricultural soils had the same diversity H'_{ARDRA} as other soil groups. The set-aside land in Sweden had not been used for five years and had not reached an equilibrium state with respect to competition between species, and thus represented higher diversity than soil of a forest, which represents a climax community. The abandoned agricultural lands studied in Latvia had not been used approximately for 15 years and thus the microbial community had progressed substantially toward a climax status.

The structure of dominant DNA fragments and consequently of the communities change at different soil depths, as shown in gel A (Fig. 3). This tendency was also observed regarding CFF; for example, in forest soils the amount of sterile mycelia proportionally increased with increasing depth. O'Brien et al. (2005) observed that mycorrhizal species predominate deeper in the soil profile whereas saprophytic species predominate in the litter layer. Further sequencing data will demonstrate if the sterile mycelia in our soil profiles was formed by mycorrhizal species.

The correlation between the number of CFU of *Trichoderma* spp. and *Trichoderma* spp. DNA amount was weak (r = 0.20). This can be explained by the fact that not all species or strains of *Trichoderma* genus can sporulate under standard laboratory conditions (Ellison et al. 1981; Schrüfer, Lysek 1990) and they may have been counted as sterile mycelia.

Distribution of cultivable microorganisms

The number of CFF and the total number of cultivable microorganisms tend to decrease with soil depth. An exception was soil profile Forest2 in which the highest number of cultivable microorganisms was at a depth of 30 – 40 cm, and soil profile Former2 in which the number of cultivable microorganisms was similar at all depths. This can be explained by the past history of profile development. In the profile Forest2 the upper 30 cm layer was composed of younger colluvial material with the original profile starting at 30 cm. The soil at Former2 was previously plaughed which destroyed the original stratification of the microorganisms.

A decrease in amounts of microorganisms in deeper soil horizons has been observed in investigations using different methods – phospholipid fatty acid analysis (Fierer et al. 2003; Fritze et al. 2000), ergosterol level (Krivtsov et al. 2007), and denaturing gradient gel electrophoresis (Krave et al. 2002). Nevertheless, some exceptions have also been described. For example, a bacterial peak has been observed at a 42.5-cm depth in the peat profile of a spruce (*Picea abies*) and birch (*Betula pubescens*) forest in Denmark (Ekelund et al. 2001) caused by partial anaerobic conditions, higher water content and higher organic matter content deeper in the soil. An even distribution of microorganisms without a decrease in their number with depth was found in a cryogenic weakly solidized loamy sandy pale soil of Yakutia (Ivanova et al. 2008). There is no permafrost in the subsoil of Latvia, but cryoturbation of the soil during winter may have some effect.

The CFF identified in our work are representatives of genera that have been isolated from a broad range of soils. For example, in the investigation of Wuczkowski et al. (2003), in forest soils of *Salix* and *Populus* stands and conventional agriculture soils the most abundant fungal genera were *Acremonium, Cladosporium, Penicillium, Cylindrocarpon* and *Trichoderma*. In boreal forest stands of *Picea mariana*, genera *Umbelopsis*, *Mortierella* and *Penicillium* were reported to be common (Summerbell 2005). In our work the most abundant genera were *Penicillium* and *Mucor* (in all analyzed soil profiles) as well as *Aspergillus* and *Trichoderma*, in some soil profiles also *Mortierella* and *Acremonium* were identified.

Quantification of fungal DNA

According to the qPCR results, fungal DNA contributed only 1.13 – 5.51% of the total extracted soil DNA (Table 3) which was several times lower amount than that found in North-Western France (Gangneux et al. 2011).

In forest lands and former agricultural lands with natural

afforestation the proportion of fungal DNA within the total soil DNA was significantly higher than in agricultural land types – 2.21 – 5.51% in forests and abandoned agricultural lands compared to 1.13 – 2.17% in currently used agricultural lands. Interestingly, the higher amount of total soil DNA of Forest1 (Fig. 1) in the upper soil horizon was not associated with a different population of fungal DNA compared to that in other forest soil samples. A strong correlation between fungal DNA amount and the period since last tillage was shown in an investigation carried out in North-Western France – the lowest fungal DNA amount was observed in fields with conventionally cultivated crops, and the highest in grasslands (Gangneux et al. 2011).

The amount of *Trichoderma* spp. DNA varied from 0.64 to 4.99 % of the total fungal DNA, and the highest relative abundance of *Trichoderma* spp. DNA was observed in agricultural soils.

Multiple linear regression analysis results (Table 4) showed that increased soil moisture content resulted in higher amounts of fungal DNA and *Trichoderma* spp. DNA. The amount of fungal DNA was affected also by land use type.

Higher fungal-bacterial DNA ratios have been previously observed in forest soil in comparison to pasture and cultivated soil (Lauber et al. 2008). In beech forest soil (Dystic Cambisol) in southern Germany the mean number of *Trichoderma* spp. ITS region copies was lower (1.2×10^6 g^{-1} of soil fresh mass) than in arable soil (Loamy Cambisol) – 1.3×10^6 g^{-1} of soil fresh mass (Hagn et al. 2003). Considering that the ITS region is amplified in fungal genome in even several 100 copies (Simon, Weiβ 2008), our calculation of *Trichoderma* genome copies (Table 3) was in the same range as the data obtained from soils in Germany.

Impact of different factors on the analyzed soil microorganisms

Multiple regression analysis showed that the number of CFF was negatively affected by increasing soil pH. In medium acid soils (forest soils – pH 4.46 – 5.30), fungal abundance was higher in comparison with neutral soils (all other soil groups – pH 6.68 – 7.90), since fungi prefer more acidic environments that bacteria. The impact of soil pH on soil microbial communities has been widely described previously. In boreal forests fungal biomass is very high in acid soil (pH < 4.1) (Högberg et al. 2007).

Soil moisture content in general has a positive impact on the number of CFF, fungal DNA amount and *Trichoderma* spp. DNA amount. Similarly, increasing moisture content was shown to cause higher biomass and *H'* index of soil fungi communities in Zoige Alpine Wetland (Feng et al. 2009).

Multiple regression showed that air temperature can have some effects on soil microbial populations. Study of the seasonality of microbial population dynamics has shown that soil microbial communities undergo a shift in function and genetic structure between winter and summer (Lipson et al. 2002).

Land use type affected the diversity of CFF, total soil DNA, fungal DNA amount and the proportion of *Trichoderma* spp. DNA. In a similar investigation in the southeastern United States in which hardwood forests, pine forests, cultivated and livestock pasture lands were compared, it was determined that the composition of fungal communities was most strongly correlated with specific soil properties (soil nutrient status) rather than land use types (Lauber et al. 2008).

We found no significant differences between fungal communities from meadows and arable lands – the diversity H'_{ARDRA} values were similar, while the proportion of fungal DNA and proportion of *Trichoderma* spp. DNA were slightly higher (not significant) in arable lands. Similarly, no substantial differences in *H'* indexes of arbuscular mycorrhizal fungi between meadows and arable lands in Central Europe were found by Oehl et al. (2003) but using other methods significant differences between soil groups were shown.

Conventional microbiological and molecular biology methods for investigating soil fungal communities showed similar results regarding relationships between depth and moisture and abundance of fungi. The numbers of CFF and the total number of cultivable microorganisms tended to decrease with increasing depth, which is in line with previous studies. There was a weak correlation between H'_{ARDRA} and H'_{CFF} diversity indexes, and the values were higher for H'_{ARDRA}. Both approaches showed higher fungal diversity at depths of 0 – 10 cm and 10 – 30 cm in comparison with a depth of 30 – 40 cm. The proportion of fungal DNA within the total soil DNA was significantly higher in forests and abandoned lands than in agricultural lands. The amount of *Trichoderma* spp. DNA was similar in all soil groups, but its proportion of the total fungal DNA amount was higher in meadows and arable lands.

An increase of land use intensity had a negative effect on the diversity of cultivable filamentous fungi (comparing forest lands with abandoned agricultural lands) and on the fungal DNA amount (comparing arable lands and meadows with forest lands and abandoned agricultural lands).

Our analysis showed that different soil types in Latvia show common features of distribution of fungal organisms, similar to the pattern found in other climatic zones and soil types. Natural phenomena (floods) and tillage causes changes in the distribution of soil fungal populations, which remain for many years. We demonstrated that molecular and conventional methods of analysis show rather weak correlation in determining total amount of soil fungi and their distribution among soil horizons. Both approaches showed the impact of soil moisture, land use type and air temperature on fungal communities. Correlation between fungal species diversity indices obtained by molecular and conventional plating methods was low. Molecular methods

gave higher diversity values, but more evenly distributed diversity of fungal species in different soil types and horizons.

The comparative analysis of fungal communities in soils under different types of natural forests, actively used and abandoned agricultural lands has provided baseline information about the fungal diversity and composition in these ecosystems although the obtained results are very variable due to the fact that each soil profile was unique in terms of soil type and land use.

Acknowledgements

The investigation was financed by the project of the European Social Fund 2004/0001/VPD1/ESF/PIAA/04/NP/3.2.3.1/0001/0001/0063. We are grateful to Prof. O. Nikodemus from the University of Latvia and to the UNDP Latvia Project "Building Sustainable Capacity and Ownership to Implement UNCCD objectives in Latvia" for the opportunity to work together. We are thankful to the company Diamedica, Ltd. for the opportunity to use the Cepheid RT-PCR analyzer.

References

Alef K., Nannipieri P. 1998. *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, San Diego. 576 p.

Barnett H.L. 1957. *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Company, Minn. 218 p.

Baudoin E., Benizri E., Guckert A. 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Appl. Soil Ecol.* 19: 135–145.

Borken W., Savage K., Davidson E.A., Trumbore S.E. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biol.* 12: 177–193.

Buyer J.S., Roberts D.P., Russek-Cohen E. 1999. Microbial community structure and function in the spermosphere as affected by soil and seed type. *Can. J. Microbiol.* 45: 138–144.

Buss K. 1997. Forest ecosystem classification in Latvia. *Proc. Latvian Acad. Sci. Sect. B* 51: 204–218.

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.

Chiarini L., Bevivino A., Dalmastri C., Nacamulli C., Tabacchioni S. 1998. Influence of plant development, cultivar and soil type on microbial colonization of maize root. *Appl. Soil Ecol.* 8: 11–18.

Clegg C.D., Lovell R.D.L., Hobbus P.J. 2003. The impact of grassland management regime on the community structure of selected bacterial groups in soil. *FEMS Microbiol. Ecol.* 43: 263–270.

Cordier C., Edel-Hermann V., Martin-Laurent F., Blal B., Steinberg C., Alabouvette C. 2006. SCAR-based real time PCR to identify a biocontrol strain (T1) of *Trichoderma atroviride* and study its population dynamics in soils. *J. Microbiol. Meth.* 68: 60–68.

Drijber R.A., Doran J.W., Parkhurst A.M., Lyon D.J. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biol. Biochem.* 32: 1419–1430.

Ekelund F., Rønn R., Christensen S. 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. *Soil Biol. Biochem.* 33: 475-481.

Ellison P.J., Harrower K.M., Chilvers G.A., Owens J.D. 1981. Patterns of sporulation in *Trichoderma viride*. *Transact. British Mycol. Soc.* 76: 441–445.

Feng S., Zhang H., Wang Y., Bai Z., Zhuang G. 2009. Analysis of fungal community structure in the soil of Zoige Alpine Wetland. *Acta Ecol. Sin.* 29: 260–266.

Fierer N., Jackson J.A., Vilgalys R., Jackson R.B. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* 71: 4117–4120.

Fierer N., Schimel J.P., Holden P.A. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem.* 35: 167–176.

Filion M., St-Arnaud M., Jabaji-Hare S.H. 2003. Direct quantification of fungal DNA from soil substrate using real-time PCR. *J. Microbiol. Meth.* 53: 67–76.

Fritze H., Pietikainen J., Pennanen T. 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. *Eur. J. Soil Sci.* 51: 565–573.

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.

Gangneux C., Akpa-Vinceslas M., Sauvage H., Desaire S., Houot S., Laval K. 2011. Fungal, bacterial and plant dsDNA contributions to soil total DNA extracted from silty soils under different farming practices: Relationships with chloroform-labile carbon. *Soil Biol. Biochem.* 43: 431–437.

Garbeva P., van Veen J.A., van Elsas J.D. 2004. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu. Rev. Phytopathol.* 42: 243–270.

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.

Grayston S.J., Wang S., Campbell C.D., Edwards A.C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* 30: 369–378.

Gregory T.R., Nicol J.A., Tamm H., Kullman B., Kullman K., Leitch I.J., Murray B.G., Kapraun D.F., Greilhuber J., Bennett M.D. 2007. Eukaryotic genome size databases. *Nucleic Acids Res.* 35: 332–338.

Grundmann G.L., Normand P. 2000. Microscale diversity of the genus *Nitrobacter* in soil on the basis of analysis of genes encoding rRNA. *Appl. Environ. Microbiol.* 66: 4543–4546.

Hackl E., Zechmeister-Boltenstern S., Bodrossy L., Sessitsch A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Appl. Environ. Microbiol.* 70: 5057–5065.

Hagn A., Wallisch S., Radl V., Munch J.C., Schloter M. 2007. A new cultivation independent approach to detect and monitor common *Trichoderma* species in soils. *J. Microbiol. Meth.* 69: 86–92.

Högberg M.N., Högberg P., Myrold D.D. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150: 590–601.

Ivanova T.I., Kuzmina N.P., Chevychelov A.P. 2008. The number of microorganisms and the microbiological activity of human-

modified cryogenic pale soils of Yakutia. *Eurasian Soil Sci.* 41: 1213–1220.

Jurgensen M.F., Richter D.L., Davis M.M., McKevlin M.R., Craft M.H. 1997. Mycorrhizal relationships in bottomland hardwood forests of the southern United States. *Wetlands Ecol. Manage.* 4: 223–233.

Kabir S., Rajendran N., Amemiya T., Itoh K. 2003. Quantitative measurement of fungal DNA extracted by three different methods using real-time PCR. *J. Gen. Appl. Microbiol.* 49: 101–109.

Kiffer E., Morelet M. 2000. *The Deuteromycetes. Mitosporic fungi. Classification and Generic Keys.* Science Publishers, Enfield. 273 p.

Klamer M., Hedlund K. 2004. Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism. *Soil Biol. Biochem.* 36: 983–988.

Kolb S., Knief C., Stubner S., Conrad R. 2003. Quantitative detection of methanotrophs in soil by novel *pmoA*-targeted real-time PCR assays. *Appl. Environ. Microbiol.* 69: 423–2429.

Kowalchuk G.A., Drigo B., Yergeau E., van Veen J.A. 2006. Assessing bacterial and fungal community structure in soil using ribosomal RNA and other structural gene markers. In: Nannipieri P., Smalla K. (eds) *Nucleic Acids and Proteins in Soil.* Springer, Berlin, pp. 159–188.

Krave A.S., Lin B., Braster M., Laverman A.M., van Straalen N.M., Roling W.F.M., van Verseveld H.W. 2002. Stratification and seasonal stability of diverse bacterial communities in a *Pinus merkusii* (pine) forest soil in central Java, Indonesia. *Environ. Microbiol.* 4: 361–373.

Krivtsov V., Brendler A., Watling R., Liddell K., Staines H.J. 2007. Some aspects of forest soil and litter ecology in the Dawyck Cryptogamic Sanctuary with a particular reference to fungi. *Acta Ecol. Sin.* 27: 813–836.

Kullman B., Tamm H., Kullman K. 2005. Fungal Genome Size Database, http://www.zbi.ee/fungal-genomesize.

Lauber C.L., Strickland M.S., Bradford M.A., Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* 40: 2407–2415.

Leckie S.E. 2005. Methods of microbial community profiling and their application to forest soils. *Forest Ecol. Manage.* 220: 88–106.

Lipson D. A., Schadt C. W., Schmidt S. K. 2002. Changes in microbial community structure and function following snowmelt in an alpine soil. *Microb. Ecol.* 43: 307–314.

Lynch J.M., Benedetti A., Insam H., Nuti M.P., Smalla K., Torsvik V., Nannipieri P. 2004. Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biol. Fertil. Soils* 40: 363–385.

Marschner P., Yang C.H., Lieberei R., Crowley D.E. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol. Biochem.* 33: 1437–1445.

McCaig A.E., Glover L.A., Prosser J.J. 2001. Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. *Appl. Environ. Microbiol.* 67: 4554–4559.

Nusslein K., Tiedje J.M. 1999. Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. *Appl. Environ. Microbiol.* 65: 3622–3626.

O'Brien H.E., Parrent J.L., Jackson J.A., Monclavo J.M., Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Appl. Environ. Microbiol.* 71: 5544–5550.

Oehl F., Sieverding E., Ineichen K., Mäder P., Boller T., Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Appl. Environ. Microbiol.* 69: 2816–2824.

Pepper I.L., Gerba C.P., Brendecke J.M. 1995. *Environmental Microbiology. A Laboratory Manual.* Academic Press, San Diego. 175 p.

Pérez-de-Mora A., Burgos P., Madejón E., Cabrera F., Jaeckel P., Schloter M. 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biol. Biochem.* 38: 327–341.

Ruisi S., Barreca D., Selbmann L., Zucconi L., Onofri S. 2007. Fungi in Antarctica. *Rev. Environ. Sci. Biotechnol.* 6: 127–141.

Schrüfer K., Lysek G. 1990. Rhythmic growth and sporulation in *Trichoderma* species: differences within a population of isolates. *Mycol. Res.* 94:124–127.

Schwieger F., Tebbe C.C. 2000. Effect of field inoculation with *Sinorhizobium meliloti* l33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) – linking of 16s rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Appl. Environ. Microbiol.* 66: 3556–3565.

Setälä H., McLean M.A. 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia* 139: 98–107.

Simon U.K., Weiß M. 2008. Intragenomic variation of fungal ribosomal genes is higher than previously thought. *Mol. Biol. Evol.* 25: 2251–2254.

Smit E., Leeflang P., Wernars K. 1997. Detection of shifts in microbial community structure and diversity in soil caused by copper contamination using amplified ribosomal DNA restriction analysis. *FEMS Microbiol. Ecol.* 23: 249–261.

Smits T.H.M., Devenoges C., Szynalski K., Maillard J., Holliger C. 2004. Development of a real-time PCR method for quantification of the three genera *Dehalobacter*, *Dehalococcoides*, and *Desulfitobacterium* in microbial communities. *J. Microbiol. Meth.* 57: 369–378.

Summerbell R.C. 2005. Root endophyte and mycorrhizosphere fungi of black spruce, *Picea mariana*, in a boreal forest habitat: influence of site factors on fungal distributions. *Stud. Mycol.* 53: 121–145.

Vanderzant C., Splittstoesser D.F. 1992. *Compendium of Methods for the Microbiological Examination of Foods,* 3rd Ed, American Public Health Association.

Wang M.-C., Liu Y.-H., Wang Q., Gong M., Hua X.-M., Pang Y.-J., Hu S., Yang Y.-H. 2008. Impacts of methamidophos on the biochemical, catabolic, and genetic characteristics of soil microbial communities. *Soil Biol. Biochem.* 40: 778–788.

Webster G., Embley T.M., Prosser J. 2002. Grassland management regimes reduce small-scale heterogeneity and species diversity of Beta-proteobacterial ammonia oxidizer population. *Appl. Environ. Microbiol.* 68: 20–30.

Wieland G., Neumann R., Backhaus H. 2001. Variation of microbial communities in soil, rhizosphere and rhizoplane in response to crop species, soil type, and crop development. *Appl. Environ. Microbiol.* 67: 5849–5854.

World Reference Base for Soil Resources. 2006. *A Framework for International Classification, Correlation and Communication*. Food and Agriculture Organization of the United Nations, Rome.

Wuczkowski M., Sterflinger K., Kraus G.F., Klug B., Prillinger H. 2003. Diversity of microfungi and yeasts and their diversity in soils of the alluvial zone national park along the river Danube downstream of Vienna, Austria („Nationalpark Donauauen"). *Die Bodenkultur* 54: 109–117.

Zachow C., Berg C., Muller H., Meincke R., Komon-Zelazowska M., Druzhinina I.S., Kubicek C.P., Berg G. 2009. Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. *ISME J.* 3: 79–92.

Received 9 February 2011; received in revised form 15 March 2011; accepted 16 March 2011