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Impact of six-year-long organic cropping on soil microorganisms and crop disease suppressiveness

Lelde GRANTINA[1], Kristine KENIGSVALDE[1], Daina EZE[1], Zaiga PETRINA[1], Ilze SKRABULE[2], Nils ROSTOKS[1], Vizma NIKOLAJEVA[1]

[1]University of Latvia
4 Kronvalda blv., Riga, Latvia
E-mail: vizma.nikolajeva@lu.lv

[2]State Priekuli Plant Breeding Institute
1a Zinatnes i., Priekuli, Priekuli distr., Latvia

Abstract

The objective of the study was to conduct a complex investigation into microbial diversity in the soil, to determine the impact of six years' long organic agricultural practices on soil microbial communities after long-term conventional cropping. Classical microbiological methods were used for analysis of cultivable microorganisms, while molecular genetics methods targeted also uncultivable organisms. During the two year period significant fluctuations were observed in the total number of analyzed groups of cultivable soil microorganisms in particular fields and at particular sampling times. On average, significantly higher numbers of all groups of analyzed cultivable microorganisms were observed in organic agriculture fields in comparison to conventional fields, e.g., the number of bacteria had increased by 70%, actinobacteria by 290%, cultivable filamentous fungi by 110%, yeasts and maltose fermenting bacteria by 190%. Results obtained by molecular methods regarding fungal diversity and *Trichoderma* spp. DNA amount did not show such an increase. In contrast to the soil microbiological indicators, plant health, in terms of plant disease suppressiveness, had not improved – crop plants in organic agriculture fields were more severely or at the same level attacked by particular plant pathogenic fungi and bacteria.

Key words: organic and conventional agriculture, microbial diversity, ARDRA, qPCR, disease suppression.

Introduction

Agricultural management, such as crop rotation, tillage, compost, manure, herbicide and synthetic fertilizer application, and water regime, are key determinants of microbial community structure in soil. Vegetation is also an important factor since plants are providing microorganisms with specific carbon sources (Garbeva et al., 2004).

In an investigation in the semiarid Canadian prairie comparing annual legumes as green manure (green fallow) with tilled fallow-wheat and continuing wheat cultivation it was estimated that after six years of green fallow practices significant improvements were detected in several microbial characteristics such as colony counts of aerobic bacteria and filamentous fungi (Biederbeck et al., 2005).

Several investigations show long term positive influence of organic farming on soil quality and microbial activity in comparison with conventional farming, due to regular crop rotation, and absence of synthetic nutrients and pesticides (Shannon et al., 2002). The diversity of bacterial functional communities has been recorded to be higher in soils from organic farms, while species diversity was similar (Liu et al., 2007). Higher abundance and diversity of actinobacteria, important decomposers of organic material, is reported in organic tomato fields than conventional ones (Drinkwater et al., 1995). The ratios of Gram+ to Gram− bacteria and of bacteria to fungi have been reported to be higher in the fields with organic treatments than in the conventional treatments (Marschner et al., 2003).

However, investigations into soil fungal communities do not clearly indicate that they are always positively influenced by organic agriculture practices. In an investigation in southern Germany it was determined by the cultivation-independent approach (molecular methods), that fungal populations were almost entirely uninfluenced by the farming management practices. Whereas active population, investigated by the isolation of hyphae using a soil-washing technique and cultivation, showed a clear response to farming management practices (Hagn et al., 2003). The propagule numbers of *Trichoderma* has been shown to be higher in soils from conventional farms (Elmholt, Labouriau, 2005; Liu et al., 2007), but it depended on the year of analyses. In an investigation in Denmark it was determined that there were no significant differences of cultivable filamentous fungi (CFF) and yeasts among organically cultivated fields and fields with synthetic fertilizer and/or animal manure. There were differences only in the abundance of particular genera of CFF – *Penicillium* spp. and *Gliocladium roseum* were more represented under organic than conventional farming (Elmholt, Labouriau, 2005).

In order to estimate the impact of agricultural practices it is important to evaluate both soil microbial parameters, and disease suppressive capacity of the soil. For agricultural purposes it is important to reduce the level of soil-borne fungal and bacterial pathogens. Disease suppressive properties of the soil depend on various factors: soil texture, structure, pH, Ca content, agricultural practices (crop rotation, tillage, fertilizers and organic amendments), soil biota (microbial activity or soil respiration, microbial community diversity and composition, population size of particular microbial groups like actinobacteria) (Postma et al., 2008).

Only small part of soil fungi (17%) (Bridge, Spooner, 2001) and bacteria (0.1–1%) (Torsvik et al., 1996) are cultivable in laboratory conditions. Therefore nowadays two approaches are used to analyze soil microbial communities – conventional plating of cultivable microorganisms and molecular methods that are independent of cultivation. Amplified rRNA gene restriction analysis (ARDRA) gives genetic fingerprinting of communities, populations or phylogenetic groups. In soil microbiology this method is used to determine diversity within phylogenetic or functional groups of microorganisms (Lynch et al., 2004). Several studies have shown that quantitative polymerase chain reaction (PCR) can be used successfully to determine the abundance of specific groups of microorganisms in soil. An important genus of soil fungi analyzed with this method is *Trichoderma* that are known for their antagonistic activities against plant pathogens (Cordier et al., 2006).

The objective of our study was to conduct a complex investigation of microbial parameters in the soil of three organic and four conventional agriculture fields in order to estimate the impact of six-year-long organic agriculture practices in Northern temperate zone conditions, and to compare the characteristics of microbial populations with crop plant health and pathogen suppression. For the characterization of soil bacteria only classical microbiological methods that analyze cultivable bacteria were used, but soil fungal populations were assessed using both classical and molecular biology methods targeting also those organisms that are uncultivable under laboratory conditions. The hypothesis was that six years of organic agriculture practices after long-term conventional agriculture can show the changes in the conditions of soil microbial populations and/or plant health and pathogen suppression.

Materials and methods

Site description. Three fields of organic agriculture (Org1, Org2, Org3) and four fields of conventional agriculture (Conv4, Conv5, Conv6, Conv7) managed by State Priekuli Plant Breeding Institute, Latvia, were examined (Table 1). They are located ca. 123 m above sea level, 57º19′ N, 25º20′ E. All analyzed fields represented sod-podzolic soil (*Luvisol, LV*).

Fields of organic agriculture have been managed as described already for six years (since 2003). The green manure is applied in each field every six years (total nitrogen 120–200 kg ha^{-1}). Additionally the amelioration of soil is achieved by cultivating clover as the improvement through the nitrogen fixation, as well as by turning the plant residues into the soil. The crop rotation in organic fields is as follows: spring crops with clover, clover, winter crops, potatoes, spring crops and crucifers for green manure. In the conventional fields, winter crops and potatoes were grown sequentially. These fields have been under long-term conventional agriculture regime since 1912.

Other soil properties of fields and crops grown for the last three years are presented in Table 1. Humus, total nitrogen, phosphorus and potassium contents were determined by the State Plant Protection Service of Latvia in 2008 in organic fields and in 2007 in conventional fields. Fertilization regime of the conventional fields is given in Table 2, while applied pesticides are listed in Table 3.

Table 1. Crop rotation and soil properties

Field	Crop			Soil texture	Humus content %	Total N kg ha^{-1}	P_2O_5 kg ha^{-1}	K_2O kg ha^{-1}
	2007	2008	2009					
Org1	clover	potato	peas, rye[a]	loamy sand	2.3	28.12	161	150
Org2	potato	crucifers[b], oil radish[a]	barley	loamy sand	1.7	27.55	150	108
Org3	winter rye	winter rye	potato	loamy sand	1.5	25.66	99	104
Conv4	winter rye	potato	barley	loam	2.7	ND[c]	158	77
Conv5	clover	winter rye	potato	loam	2.3	ND[c]	184	126
Conv6	annual rye-grass	barley with vetch	beans	loamy sand	1.9	ND[c]	48	80
Conv7	oats	spring oilseed rape	barley	loam	2.3	ND[c]	115	102

[a] – green manure, [b] – mixture of oil radish, oil seed rape and mustard, [c] – not determined

Table 2. Fertilization of the conventional fields

Field	N:P:K in 2008	Amount kg ha^{-1}	N:P:K in 2009	Amount kg ha^{-1}
Conv4	11:9:20	500	6:15:30 additional N	350 70
Conv5	16:18:14 additional N	200 200	17:10:14	330
Conv6	without fertilization	0	without fertilization	0
Conv7	6:15:30 additional N surface fertilizing 18:18:18	100 100 5	without fertilization	0

Table 3. Pesticides applied in the conventional fields

Field	Pesticides used in 2008	Amount per hectare	Pesticides used in 2009	Amount per hectare
Conv4	herbicide Zenkor fungicide Ridomil Gold fungicide Gloria	0.4 kg 2.5 kg 2.0 kg	herbicide Sekator	0.15 l
Conv5	herbicide Granstar Preiss	3.0 kg	herbicide Mistral insecticide Fastak herbicide Pantera fungicide Ridomil Gold fungicide Gloria fungicide Penkoceb	0.3 kg 0.2 kg 1.5 kg 2.0 kg 2.0 l 2 kg
Conv6	herbicide Bazagran herbicide Stomp	1 l 2 l	herbicide Bazagran herbicide Stomp	1 l 2 l
Conv7	herbicide Treflan herbicide Lontrel insecticide Fastak	4 l 0.3 l 0.4 l	herbicide Sekator	0.15 l

The total amount of precipitation 30 days before soil sampling was analyzed using raw data obtained from the data base of Latvian Environment, Geology and Meteorology Centre. Meteorological station in Priekuli was chosen for its proximity to the fields.

Soil sampling and estimation of pH and moisture content. Soil samples were taken in June and August 2008 and 2009. Nine subsamples were collected on a transect of each field at a depth of 10–15 cm (three subsamples in each corner of the field and three subsamples in the middle of the field, 100 g each). The subsamples were pooled together to create three larger samples for every field. Altogether 84 soil samples were analyzed. Samples were placed in sterile plastic bags "Whirl-Pak" ("Nasco", USA), stored at +4°C for a few days for the enumeration of cultivable microorganisms and after were stored at −20°C. The pH of the soil samples was measured in distilled water according to the ISO 10390 method. The moisture content of the soil samples was determined according to the ISO 11465 method.

Microbial parameters. In order to estimate the number of colony forming units (CFU) of cultivable microorganisms by a plate count method soil sample dilutions were prepared by adding 10 g of soil to 90 ml of sterile distilled water. Each soil sample was analyzed in two replicates. Suspensions were homogenized 1 h on a horizontal shaker. After that serial dilutions were prepared, and 0.1 ml of dilutions 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were used. Agarised malt extract ("Biolife", Italy) was used for cultivation of cultivable filamentous fungi (CFF), yeasts and particular groups of bacteria. The number of CFU was assessed after three days' incubation at 20 ± 2°C. The number of bacterial CFU was estimated on yeast extract peptone agar "Nutrient broth E" ("Int. Diagnostics Group", Great Britain). The number of CFU was assessed after three days' incubation at 20 ± 2°C. Number of CFU was expressed per gram of dry soil. Since actinobacteria represent an important group of soil bacteria, they were quantified separately.

Genera of filamentous fungi were determined after 10 days of incubation of the fungal CFU plates on malt extract agar according to morphological characteristics and light microscopy using keys (Barnett, 1957; Kiffer, Morelet, 2000).

In order to monitor plant health each growing season the information about the time of outbreak and severity of late blight (*Phytophthora infestans*), potato scab caused by *Streptomyces scabies* and black scurf of potato caused by *Rhizoctonia solani* was recorded.

Analyses of soil microbial diversity. DNA extraction. Total soil DNA was extracted using the PowerSoil™ DNA isolation kit ("MO BIO Laboratories", USA). Soil samples (250 mg) were homogenized using horizontal "Mixer Mill Type MM 301" ("Retsch", Germany) at a maximal speed of 30 Hz (1800 oscillations min^{-1}) for 10 minutes. DNA was extracted twice from each sample and all DNA extracts from the same field were pooled. The amount and purity of the DNA was determined spectrophotometrically using "Ultrospec 3100 Pro" ("Amersham Biosciences", Sweden) and by a 1% agarose 1 × TAE (w v^{-1}) gel electrophoresis.

Amplification with PCR. For ARDRA analyses of soil DNA the fungal nuclear ribosomal RNA 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) that are specific to the ITS1-5.8S-ITS2 region in *Ascomycota*, *Basidiomycota* and *Zygomycota* fungi. PCR reactions were carried out in 50 µl volume in an "Eppendorf Mastercycler Personal" ("Eppendorf", Germany). The reaction contained 2 u Hot Start *Taq* DNA polymerase, 5 µl 10X Hot Start PCR buffer, 0.2 mM dNTP, 2 mM $MgCl_2$, 0.75 µl bovine serum albumin 20 mg ml^{-1} (all reagents from "Fermentas", Lithuania), 0.5 µM of each primer ("Operon Biotechnologies", Germany), and 1 µl of DNA template. The PCR conditions were as follows: initial denaturation step of 4 min at 95°C, 40 s of denaturation at 95°C, 40 s of annealing at 52°C, 1 min of primer extension at 72°C (30 cycles) and final extension 10 min at 72°C.

After the PCR the amplification products were precipitated by addition of 450 µl of mixture containing 96% ethanol and sodium acetate (3 M, pH 5.0) in the ratio 19:1. The precipitated DNA was divided into two parts and digested with restriction endonuclease *Bsu*RI (Chabrerie et al., 2003) ("Fermentas", Lithuania). Restriction products were visualized in 6% polyacrylamide gel electrophoresis in two replicates. Gels were photographed with a "BioSpectrum AC Imaging System" and analyzed with software *Kodak 1D*. For the estimation of the Shannon-Weaver diversity index the following equation was used:

$H' = -\sum p_j \log_2 p_j$, where p_j = relative intensity of individual band (Gabor et al., 2003).

We calculated also the species richness that corresponds to the total number of distinct bands (number of operational taxonomic units, OTUs) (Gabor et al., 2003) in an ARDRA profile.

Quantitative real time PCR (qPCR). For the qPCR primers uTr and uTf specific to *Trichoderma* spp. were used (Hagn et al., 2007). Each soil sample was analyzed in three replicates. The reactions were carried out in 25 µl volume. The mixture contained 12.5 µl SYBR® Premix Ex Taq ("TaKaRa", Japan) for the year 2008 samples or Maxima™ SYBR Green qPCR Master Mix 2x ("Fermentas", Lithuania) for the year 2009 samples, 1 µM of each primer, 9.5 µl of sterile distilled water and 1 µl of the DNA template. The reaction conditions in "SmartCycler" ("Cepheid", USA) were the following: initial denaturation step of 60 s at 95°C, 40 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 60°C, 30 s of primer extension at 72°C. Serial dilutions of DNA from a pure culture of *Trichoderma harzianum* (MSCL 309) was used to construct the standard curve (separate for each SYBR Green kit).

Ribosomal RNA gene sequencing. For sequencing of the fungal ribosomal RNA gene region the genomic DNA of 39 isolates representing dominant filamentous fungi and sterile mycelia was amplified with primers ITS1F and ITS4, while representatives from genera *Penicillium* and *Aspergillus* were amplified also with modified fungal β-tubulin primers T12 (5'-TCT GGA TGT TGT TGG GAA TCC-3') and T22 (5'-AAC AAC TGG GCC AAG GGT CAC-3'), originally developed by O'Donnell and Gigelnik (1997). Both strands of the amplification products were sequenced with "BigDye Terminator v3.1 Cycle Sequencing Kit" ("Applied Biosystems", USA). Obtained sequences were analyzed using *Staden Package 1.6.0* release. The resulting consensus sequences were used in BLASTN homology search against the NCBI nucleotide database. Species were determined combining information from the most homolog sequence in the database and light microscopy results using keys (Barnett, 1957; Kiffer, Morelet, 2000).

Statistical analyses. Significance of differences between means was analyzed by the Tukey-Kramer test ($\alpha = 0.05$). Correlation analyses were done with *Excel* ("Microsoft", USA). Multiple regression analysis was done with *R package* (http://www.R-project.org) to evaluate the influence of several factors – soil moisture content, soil pH, precipitation, soil nutrient content and crop. In order to determine the impact of the crop on the microbial diversity, the crops were ranked as follows: 1) potatoes, 2) spring rape, 3) winter rye, 4) barley, 5) barley with vetch, 6) beans, 7) crucifers, 8) peas and rye (green manure).

Results and discussion

Soil chemical and physical parameters. The total amount of rainfall during the 30 days preceding the soil sampling was as follows: 83.80 mm in June 2008, 63.20 mm in August 2008, 20.60 mm in June 2009 and 141.50 mm in August 2009. Slight differences in soil moisture content were found at particular sampling times – 14.2 ± 2.9% in June 2008, 18.4 ± 1.2% in August 2008, 21.7 ± 1.2% in June 2009 and 14.3 ± 2.2% in August 2009.

Soil pH did not change significantly during the analyzed two year period – it ranged from 6.60 ± 0.41 to 6.82 ± 0.21 and it did not differ significantly between the organic and conventional cropping systems. Similarly, other parameters such as humus content, potassium and phosphorus content on average was similar in both groups of fields, the exceptions were fields Org3 and Conv6 with reduced level of phosphorus and fields Conv4 and Conv6 with reduced level of potassium (Table 1). The impact of various elements content on the analyzed microbial parameters is discussed later.

Soil microbial parameters. During the two year period, the level of cultivable bacteria was constant in the field Conv4, while in other fields marked changes were observed, e.g., substantial increase of bacterial CFU were detected in fields Org1 and Org2 in the August of 2009 (Fig. 1a). However, the results of Tukey-Kramer test showed that these differences were not statistically significant. Significant differences were found in the field Conv5 comparing both sampling years – in 2009 the number of bacterial CFU was increased 2.14 times ($F_{5, 5} = 11.16$, $P = 0.007$). The comparison of log CFU data showed that on average the total number of bacteria was significantly higher in organic agriculture fields in comparison with conventional fields ($F_{35, 47} = 6.23$, $P = 0.02$). The number of bacteria in organic fields exceeded that in conventional fields by 70%.

There was a tendency that at the end of the summer in year 2008 the number of actinobacteria in all fields decreased (except field Org2) but in year 2009 – in all fields increased (Fig. 1b) but these changes were not statistically significant. According to the Tukey-Kramer test the total number of actinobacteria was significantly higher in organic agriculture fields ($F_{35, 47} = 6.07$, $P = 0.02$) – on average almost four times if comparing results of two years.

Total number of yeasts and maltose utilizing bacteria was fluctuating during research period and on average it was higher in samples of the year 2009 (Fig. 1d) and also in organic agriculture fields in general in comparison to conventional fields – on average by 190% (statistically not significant).

The ratio of bacteria to fungi differed significantly in particular sampling times (Fig. 2). On average the ratio of bacteria to fungi was significantly higher in the conventional fields (498 *v.s.* 312; $F_{35, 47} = 5.29$, $P = 0.024$), which is in line with previously published investigation of old mining sites in Germany. In this investigation, higher ratio of bacteria to fungi determined by signature phospholipid fatty acids was detected in the fields with long term organic treatments (fertilization with manure, sewage sludge or straw) in comparison with conventional treatments (NPK mineral fertilizer treatment with all plant residues removed; mineral fertilizer treatment with plant residues incorporated into the soil). These results were explained by differences in organic matter composition and corresponding substrate availability among different treatments (Marschner et al., 2003).

A common tendency was observed that total number of CFF was increased in the year 2009 in all fields with exception of field Conv7 but these differences were statistically significant only in fields Org1 ($F_{5, 5} = 11.98$, $P = 0.006$) and Org2 ($F_{5, 5} = 21.39$, $P = 0.0009$). The explanation, why the total number of CFF increased significantly in the second year in almost all fields, is still lacking, since none of the factors included in the regression models explained this shift. In spite of the fact that field Conv5 received fungicides (mancozeb and others) several times during the second summer (Table 3) the total number of CFF was increased 9.5 times at the end of the August 2009 in comparison with previous level (Fig. 1c). Data about dominating CFF genera showed that

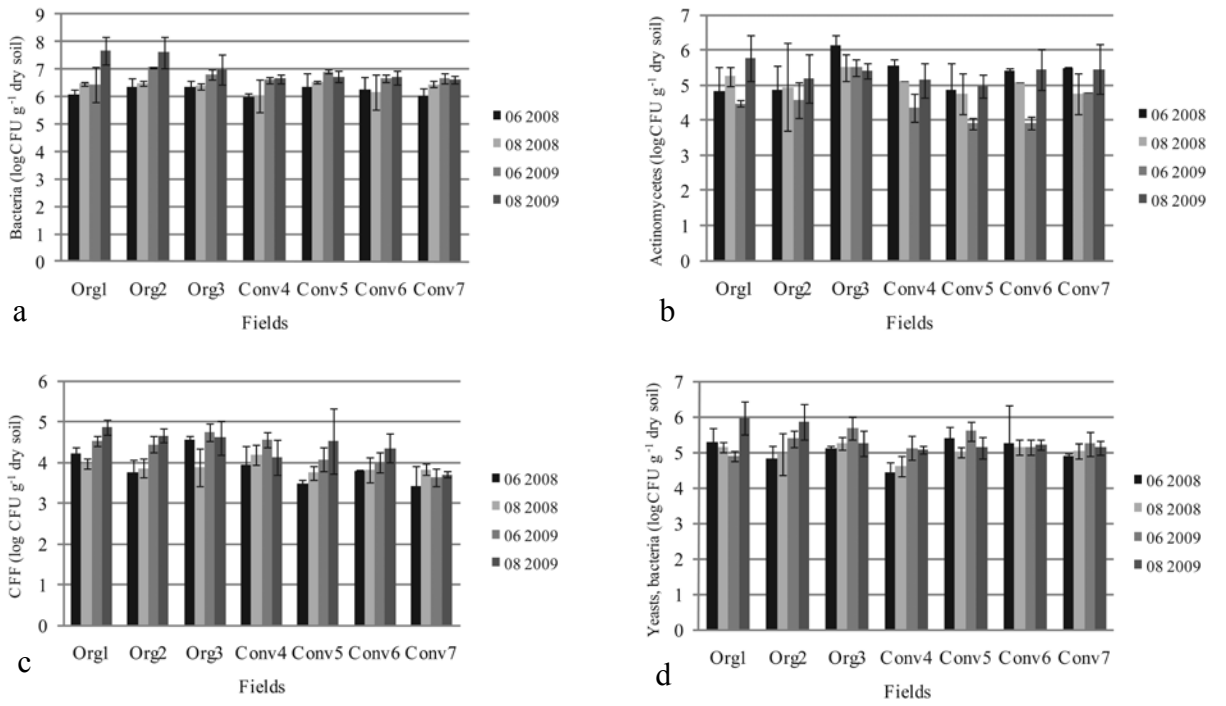


Figure 1. Total number of cultivable microorganisms (± S.D.) in each field at four sampling times (n = 6): a) total number of bacterial CFU, b) total number of CFU of actinobacteria, c) total number of cultivable filamentous fungi (CFF), d) total number of yeasts and maltose utilizing bacteria

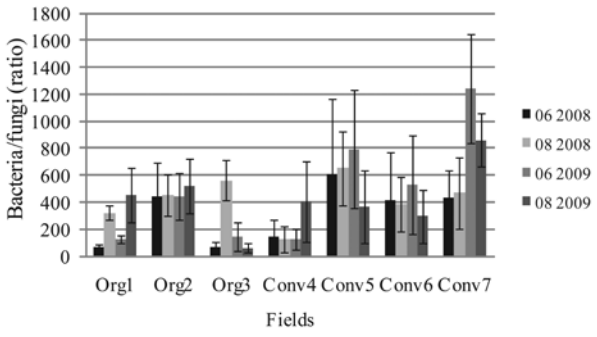


Figure 2. Ratio of bacteria to fungi (± S.D.) in each field at four sampling times (n = 6).

especially the number of CFU of *Mucor* spp. and sterile mycelia was increased in the year 2009, while CFU of other genera remained unchanged. It contradicts other investigations which found that application of such fungicide as mancozeb in amount 10 mg kg^{-1} soil decreased the amount of fungi for at least 3 months (Doneche et al., 1983), although the concentration of mancozeb applied on the Conv5 field was significantly lower (Table 3). In general, total number of CFF was significantly higher in organic fields ($F_{35, 47} = 1.40$, $P = 0.009$). The increase of CFF numbers in organic agriculture fields was on average approximately by 110%.

Changes in abundance of dominant fungal genera (*Trichoderma*, *Mucor*, *Mortierella*, *Penicillium* and *Verticillium*) and sterile mycelia (not sporulating after 10 days of incubation) were evaluated in the two year period. Similarly to the investigations of Liu et al. (2007), in our study there were no statistically significant differences in the propagule numbers of *Trichoderma* genus among fields of organic and conventional agriculture. It is assumed that *Trichoderma* spp. are less affected by a soil disturbance (after the use of pesticides) than other soil fungi, and are able quickly to colonize niches left by other organisms in conventional fields (Liu et al., 2007). The most abundant genus was *Penicillium* – on average 37.84 ± 14.35% of all fungi, while other genera were represented by 5–10% of all CFF and sterile mycelia covered 32.97 ± 10.12%. In organic fields only propagule numbers of *Penicillium* ($F_{11, 15} = 5.74$, $P = 0.02$) and *Verticillium* ($F_{11, 15} = 5.16$, $P = 0.03$) were significantly higher than in conventional fields ($p < 0.05$). Higher numbers of *Penicillium* in organic fields have been recorded in the work of Elmholt and Labouriau (2005). Other genera were evenly abundant in both groups of fields. Average numbers of the five mentioned genera and sterile mycelia are given in Figure 3.

In June 2008, representatives from such less abundant genera as *Absidia* (field Org3), *Cephalosporium* (Org2) and *Botrytis* (Org3) were detected in small numbers. In June 2009, *Geomyces* spp. were isolated from all fields (1.11 ± 0.56) x 10^3 g^{-1} of dry soil) and *Staphylotrichum* spp. from the fields Conv4 and Conv5 in small numbers. *Fusarium* spp. and *Acremonium* spp. were isolated from the samples of August 2009 (Org1, Org3, Conv7 and Org1, Org2, Org3, Conv5, Conv6, respectively).

Consequently, in our investigation we found that colony counts of all groups of cultivable microorganisms (bacteria, actinobacteria, yeasts and maltose utilizing bacteria and CFF) were significantly higher in organic agriculture fields after six years of organic cropping than in continuous conventional fields (Fig. 4). This is in line with the results of Biederbeck et al. (2005) in the semiarid Canadian prairie where numbers of bacteria and filamentous fungi were several times higher in fields with partial fallow and with annual legumes as green manure in comparison to fields with tilled fallow – wheat

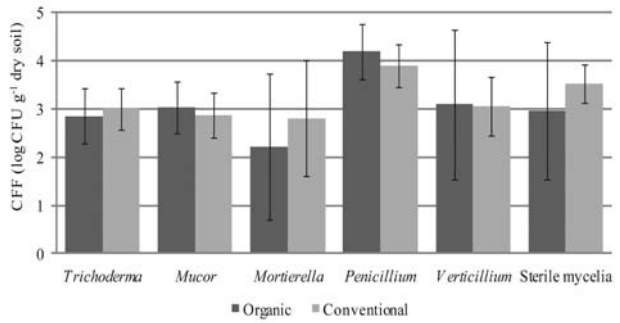


Figure 3. Average numbers (± S.D.) of the most abundant filamentous fungi genera and sterile mycelia (n = 72 for organic agriculture fields, n = 96 for conventional agriculture fields)

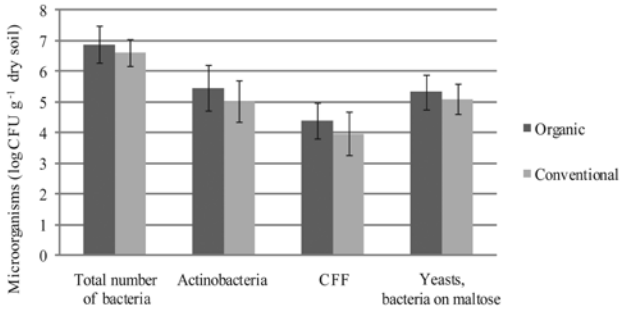


Figure 4. Average numbers (± S.D.) for two year period of all analyzed groups of cultivable microorganisms in organic and conventional fields (n = 72 for organic agriculture fields, n = 96 for conventional agriculture fields)

cropping system after six years of treatments. This investigation also proved that the microbiological attributes of the soil were sensitive and responsive to the beneficial influence of the particular cropping systems. Similarly, 1.6 times higher bacterial numbers under low input agriculture in comparison with high input agriculture have been recorded in an eight-week microplot experiment in the Netherlands (Bloem et al., 1992).

Organic agricultural practices are more favourable for soil microorganisms because they return more organic residues to the soil, and most of the N remaining in soil after the incorporation of green manure increases the mineralizable fraction of soil N. Such microbial population increases may be short-term or persist for at least one year after green manure incorporation (Biederbeck et al., 2005).

Soil microbial diversity. Examples of ARDRA results in 6% polyacrylamide gel electrophoresis are given in Figures 5a and 5b. Average results of the fungal diversity indices *H'* and number of OTUs are given in Figures 5c and 5d, respectively. There were no statistically significant differences among fields of organic and conventional agriculture, although the mean diversity index *H'* was higher in the organic fields in comparison to the conventional agriculture fields (2.56 *v.s.* 2.43). The numbers of OTUs obtained from the organic fields were also higher than from the conventional fields (7.71 *v.s.* 7.66). Similarly to our study, no significant differences were detected between the two agricultural regimes regarding number of phylotypes per field and Shannon diversity indices of arbuscular mycorrhizal fungi in onion fields in Netherlands using molecular methods (Galván et al., 2009).

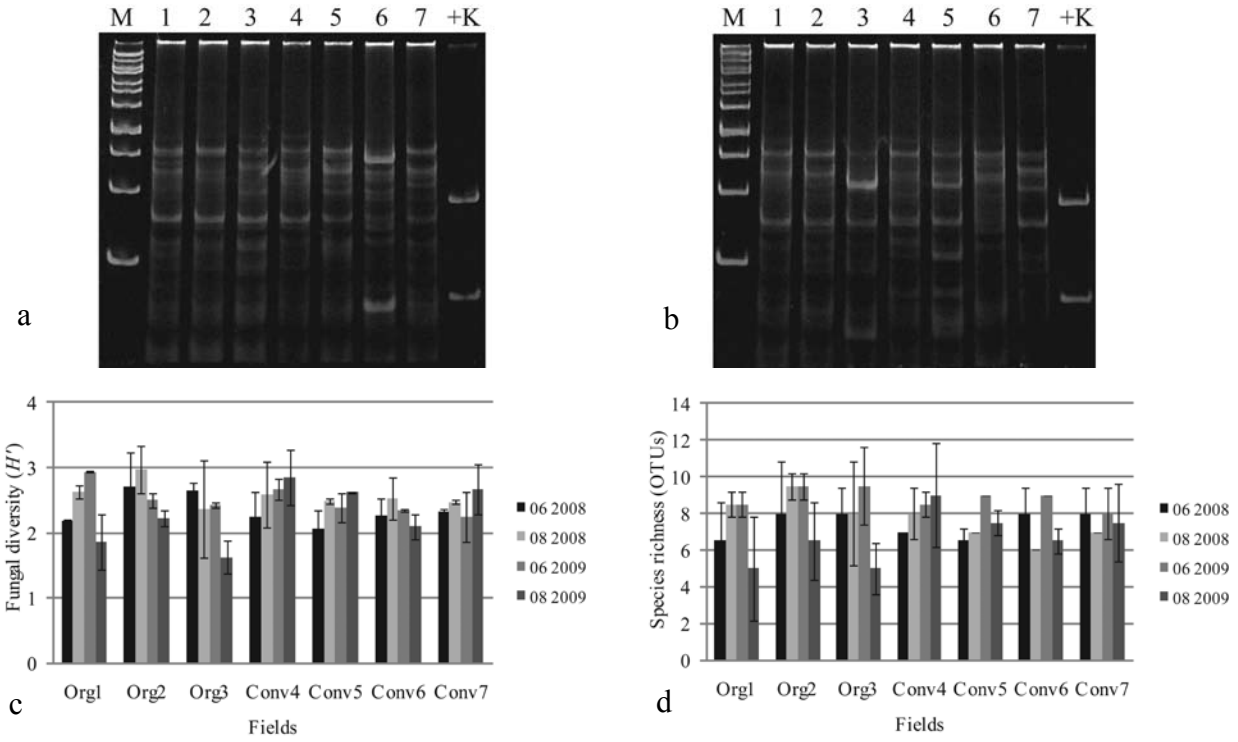


Figure 5. Results of ARDRA in 6% polyacrylamide gel electrophoresis: a) June 2008, lanes 1–3 organic agriculture fields, 4–7 conventional agriculture fields; b) August 2009, lanes 1–3 organic agriculture fields, 4–7 conventional agriculture fields; M – GeneRuler 1kb DNA Ladder ("Fermentas", Lithuania), +K – restriction results of *Trichoderma rossicum* DNA; c) Shannon-Weaver diversity index *H'* (± S.D.) of fungi in all analyzed fields at four sampling times (n = 2); d) number of OTUs (± S.D.) of fungi in all analyzed fields at four sampling times (n = 2)

qPCR indicated an increase in the amount of *Trichoderma* spp. DNA in 2009, especially in August, in fields Org1, Org2, Org3, Conv6 and Conv7 (Fig. 6). However, there were no statistically significant differences among fields of organic and conventional agriculture, although the mean values of this parameter were higher in organic fields – 9.23 ng g^{-1} dry soil *v.s.* 7.17 ng g^{-1} dry soil.

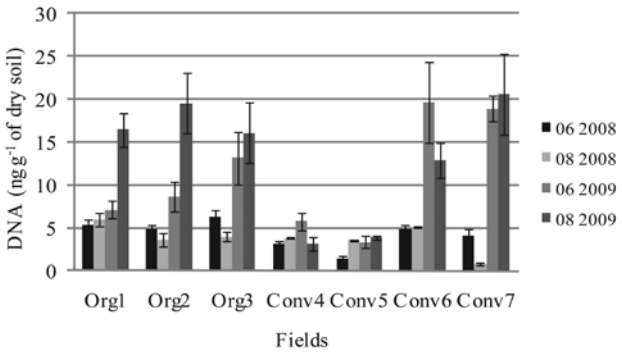


Figure 6. The amount of *Trichoderma* spp. DNA (± S.D.) in all analyzed fields at four sampling times (n = 3)

Significant part of all CFF was isolated as sterile mycelia. Sequencing showed that isolated sterile mycelia belonged to various fungal genera – *Bionectria*, *Stephanonectria*, *Arthrinium*, *Verticillium*, *Trichocladium*, *Phoma* and *Acremonium*. Two isolates remained unidentified also after sequencing. List of all sequenced fungal isolates is given in Table 4. One strain of each species was deposited in Microbial Strain Collection of Latvia (MSCL).

Sequencing enabled identification of fungal isolates to the species level with higher probability than according only to the morphological characteristics. Identified fungi mostly are saprophytic, but several plant pathogenic fungi were also detected, e.g., from the barley field (Org2) *Fusarium oxysporum* was isolated that causes root rot on barley as well as on other cereals and is common in Latvia (Treikale et al., 2008). From the field Conv4, where potatoes were grown in 2008, *Verticillium dahliae* was isolated that causes wilting in many plants and is recorded to be pathogenic also for potato (Kapsa, 2008). *Phoma eupyrena* that causes dry tuber rot of potato (Choiseul et al., 2007) was isolated from the field Conv5 planted with potato in 2009.

Aspergillus fumigatus, *Humicola grisea*, *Acremonium* sp., *Cladosporium cladosporioides*, *Mucor hiemalis*, *Penicillium canescens* and *Trichoderma hamatum* were isolated from those fields in our investigation, where rye has been cultivated within the last three years as crop or as green manure similarly to the study in Poland, where rye was used as a cover crop in tomato fields (Jamiołkowska, Wagner, 2005).

Results of statistical analyses. Multiple regression analyses showed that higher soil moisture content, for example, 21.7 ± 1.2% in June 2009, had significant negative impact on the total number of actinobacteria, while it had a positive effect on the number of sterile mycelia and species richness OTUs obtained by ARDRA method (Table 5). The total amount of precipitation in the 30 days preceding sampling significantly negatively affected the ratio of bacteria to fungi and the number of *Mortierella* spp.

According to the multiple regression analyses, the soil humus content (Table 1) had a negative impact on the total number of bacteria, total number of CFF and number of cultivable *Penicillium* spp. (Table 5). In the case of CFF, it contradicts the results of Hr elová et al. (1999), where the impact of the organic matter (oxidizable carbon) on soil microfungi was positive, but in the case of total number of bacteria and number of cultivable *Penicillium* spp. the observed impact was similar. Negative impact of humus content on soil fungi has been recorded in the work of Marschner et al. (2003).

Available phosphorus (P_2O_5) in the soil (Table 1) had positive impact on the total number of bacteria. In other investigations, positive impact of phosphorus was observed on soil saprophytic microfungi (Hršelová et al., 1999) but negative on species richness of arbuscular mycorrhizal fungi in soil macrocosms (Huang et al., 2005). Such contradiction can be explained by the fact that the numbers of phosphate solubilizing microorganisms vary from soil to soil (Gyaneshwar et al., 2002).

Available potassium (K_2O) in the soil (Table 1) had positive influence on the total number of CFF and yeasts and maltose utilizing bacteria. The nitrogen content in the fertilizer had negative impact on the total number of CFF but positive impact on the ratio of the bacteria to the CFF (Fig. 7). Negative impact of the phosphorus content in the fertilizer was observed on the total number of actinobacteria and on the amount of *Trichoderma* spp. DNA (data not shown). Positive impact of the potassium content in the fertilizer was observed on the total number CFF and on the fungal diversity estimated by ARDRA in agarose gels (data not shown).

Results of multiple regression analysis showed that soil pH did not affect significantly any of the analyzed microbial parameters. Several parameters (total numbers of CFU of *Trichoderma*, *Mucor*, *Verticillium*, sterile mycelia, and OTUs in agarose gels) were not affected by any of the chosen factors (not shown in Table 5).

From all factors that were different in both agricultural practices, only the use of artificial fertilizers was included in the regression models. Impact of such important factors as the use of pesticides and frequency of ploughing was not evaluated mathematically, although generally no negative effects of pesticides or ploughing intensity on the microbial numbers and diversity were observed. The frequency of ploughing depends not only on the chosen agricultural regime but also on the crop, and the crop according to the ranks applied in statistical analysis significantly affected only the total number of soil bacteria and in the case of potatoes – the CFU number of sterile mycelia. Crops that had potentially promotive effect on soil microorganisms (green manure, growth of legumes that increases the amount of nitrogen in the soil etc.), generally increased the number of bacteria.

Crop pathogens. In 2008, the first damage of the late blight (*Phytophthora infestans*) in organic fields was observed 7–10 days earlier than in conventional fields. Late blight significantly destroyed foliage (30–100%) in organic fields 10 to 14 days before it reached such level in conventional fields. In 2009, the first spots of the disease on potato leaves were observed at the same time on both environments, but significant foliage damage (5–100%) was assessed after 10 days in organic fields and only after 24 days in conventional fields. The application of fungicide delayed the late blight development in conventional fields and prolonged vegetation period for longer time. The late blight development was faster in 2008 than in 2009 due to more favourable weather conditions (more rainfall during August) in 2008. The rainfall in August 2009 was half of that for the same period in two previous years.

Table 4. Fungal isolates in organic and conventional fields

Field	Species	Amplified gene	Homolog sequence in NCBI	Max identity %	Strain in MSCL
Org1	*Penicillium canescens*	rDNA	FJ439586. 1	99	879
Org1	*Penicillium commune* *Penicillium crustosum*	rDNA β-tubulin	GQ458026. 1 FJ012877. 1	99 98	878
Org1	*Aspergillus fumigatus* *Aspergillus fumigatus*	rDNA β-tubulin	GQ461905.1 AY048754.1	99 99	870
Org1	***Bionectria ochroleuca***[a]	rDNA	AF106532.1	99	886
Org1	*Trichoderma rossicum*	rDNA	EU280089.1	99	883
Org2	*Penicillium griseofulvum* *Penicillium aurantiogriseum*	rDNA β-tubulin	DQ339570.1 FJ012878.1	99 95	874
Org2	Uncultured ascomycete[b]	rDNA	EU520630. 1	99	–
Org2	*Fusarium oxysporum*	rDNA	EU364863. 1	99	–
Org2	*Penicillium pinophilum*	rDNA	GU566216.1	98	880
Org2	***Bionectria ochroleuca***	rDNA	AF106532.1	99	–
Org2	*Penicillium canescens* *Penicillium crustosum*	rDNA β-tubulin	FJ439586.1 FJ012877.1	99 92	879
Org2	*Cladosporium cladosporioides*	rDNA	GQ458030.1	100	864
Org3	*Mucor hiemalis*	rDNA	EU326196.1	99	–
Org3	*Penicillium canescens* *Penicillium aurantiogriseum*	rDNA β-tubulin	FJ439586.1 FJ012878.1	99 91	875
Org3	***Stephanonectria keithii***	rDNA	EU273554.1	96	866
Org3	*Trichoderma hamatum*	rDNA	GQ220703.1	98	867
Org3	*Amylomyces rouxii*	rDNA	AY238888.1	99	865
Org3	*Cladosporium macrocarpum*	rDNA	EF679380.1	100	887
Org3	***Arthrinium sacchari***	rDNA	AF393679.2	99	881
Conv 4	*Trichoderma rossicum*	rDNA	EU280089.1	99	–
Conv 4	*Humicola grisea*	rDNA	AY706334.1	100	868
Conv 4	*Aspergillus fumigatus*	rDNA	AY214448.1	100	–
Conv 4	*Talaromyces ucrainicus*	rDNA	AY533694.1	99	885
Conv 4	*Penicillium aurantiogriseum* *Penicillium aurantiogriseum*	rDNA β-tubulin	AJ005488.1 FJ012878.1	100 99	–
Conv 4	***Verticillium dahliae***	rDNA	DQ282123.1	97	863
Conv 4	*Penicillium melanoconidium*	rDNA	AJ005483.1	100	871
Conv 4	***Trichocladium asperum*** Uncultured soil fungus	rDNA rDNA	AM292050.1 DQ420780.1	99 99	–
Conv 4	*Geomyces destructans*	rDNA	EU854572.1	98	884
Conv 4	*Paecilomyces marquandii*	rDNA	GU566261.1	98	–
Conv 5	***Phoma eupyrena***	rDNA	AJ890436.1	100	–
Conv 5	*Penicillium commune* *Penicillium allii*	rDNA rDNA	EU833216.1 AJ005484.1	99 99	–
Conv 5	***Acremonium* sp.**	rDNA	AJ890439.1	99	–
Conv 5	**Uncultured ascomycete**	rDNA	AY833028.1	96	–
Conv 5	**Uncultured soil fungus**	rDNA	EU826895.1	99	873
Conv 6	*Acremonium* sp.	rDNA	AJ890439.1	99	–
Conv 6	*Paecilomyces marquandii*	rDNA	GU566261.1	99	888
Conv 6	*Penicillium verruculosum*	rDNA	AF510496.1	97	882
Conv 6	*Paecilomyces carneus*	rDNA	EU553305.1	98	887
Conv 6	*Paecilomyces carneus*	rDNA	AB258369.1	99	872

[a] – isolates with names in bold were isolated as sterile mycelia, [b] – *Scopulariopsis* sp. according to microscopy results

The prevalence of potato scab caused by *Streptomyces scabies* and black scurf of potato caused by *Rhizoctonia solani* was similar in the fields of both agricultural practices.

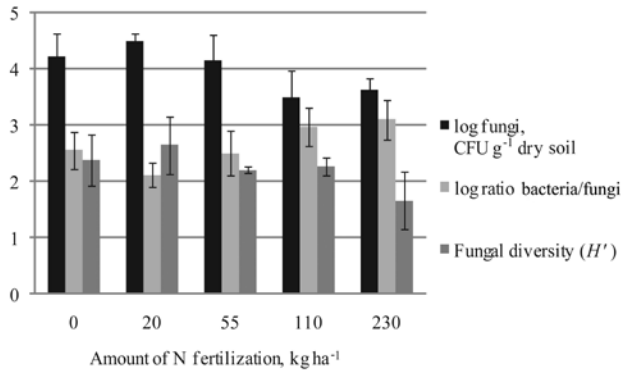
Consequently, in contrast to the soil microbiological indicators that showed improvement after six years of organic cropping in comparison to the conventional agricultural fields, the plant health, in terms of plant disease suppression, was been improved. Controversial results about the capacity of minimum tillage and organic agriculture systems to reduce the disease levels, for example, of common root rot of cereals caused by *Cochliobolus sativus*, *Verticillium* wilt and common scab of potato, have been obtained in previous investigations (Bailey, Lazarovits, 2003). One of the reasons for heavier infection of potato with *Phytophthora infestans* in organic fields could be the fact that soil can act as a reservoir of the inoculum of pathogenic fungi, for example oospores of late blight *Phytophthora infestans* can survive in the soil in the absence of the host for several years (Drenth et al., 1995).

Fungal activity measured as fungal biomass has been proved to correlate with *R. solani* suppression in soil (Postma et al., 2008). Our investigation shows that increase in the number of CFF does not result in the disease suppression. One of the explanations is that six years is a too short time period to reduce the plant pathogen levels in the soil because the crop rotation has gone through the whole cycle only one time.

Table 5. Results of multiple regression analysis

Factor	CFF	*Mortierella*	Sterile mycelia	Yeasts and bacteria	Bacteria	Actinobacteria	Ratio bacteria to fungi	*Trichoderma* spp. DNA	*H'*	OTUs
Soil moisture	NS[a]	NS	0.02	NS	NS	<0.001[b]	NS	NS	0.005	<0.001
Total precipitation	NS	0.03[b]	NS	NS	NS	NS	0.03[b]	NS	NS	NS
Soil humus content	0.01[b]	NS	NS	NS	0.04[b]	NS	NS	NS	NS	NS
P_2O_5	NS	NS	NS	NS	0.006	NS	NS	NS	NS	NS
K_2O	0.003	NS	NS	0.005	NS	NS	NS	NS	NS	NS
N content in fertilizer	0.01[b]	NS	NS	NS	NS	NS	0.0009	NS	NS	NS
P content in fertilizer	NS	NS	NS	NS	NS	0.0007[b]	NS	0.005[b]	NS	NS
K content in fertilizer	0.002	NS	NS	NS	NS	NS	NS	NS	NS	NS
Crop	NS	NS	NS	NS	0.035	NS	NS	NS	NS	NS
Multiple R^2	0.51	0.18	0.19	0.33	0.37	0.51	0.43	0.27	0.27	0.47
p	0.002	0.03	0.02	0.007	0.01	0.0002	0.009	0.005	0.005	<0.001

[a] – not significant, [b] – negative impact



Note. Without nitrogen in the mineral fertilization – fields Org1, Org2, Org3, Conv6 in both years and Conv7 in 2009; 20 kg ha^{-1} – field Conv4 in 2009; 55 kg ha^{-1} – fields Conv4 in 2008 and Conv5 in 2009; 110 kg ha^{-1} – Conv7 in 2008; 230 kg ha^{-1} – Conv5 in 2008.

Figure 7. The impact of the nitrogen content in the mineral fertilizer on the total number of CFF, ratio of the bacteria to the CFF and fungal diversity index *H'* (± S.D.)

Conclusions

1. After six years of organic cropping practice, significantly higher numbers of all groups of cultivable microorganisms (bacteria, actinobacteria, yeasts and filamentous fungi) were observed in organic agriculture fields in comparison to conventional fields. In the case of filamentous fungi in organic fields propagule numbers of *Penicillium* and *Verticillium* were significantly higher. There were no statistically significant differences in the propagule numbers of *Trichoderma* and other dominant filamentous fungi genera (*Mucor*, *Mortierella*, *Absidia*, *Cephalosporium*, *Geomyces*, *Staphylotrichum*, *Fusarium*, *Acremonium*) among fields of organic and conventional agriculture.

2. Results obtained by molecular methods regarding fungal diversity and *Trichoderma* spp. DNA amount did not show any significant difference among fields of both agricultural managements although the tendency for organic fields to have higher values of both parameters was observed.

3. Soil filamentous fungi identified by microscopic and molecular methods were mostly saprophytic, but several plant pathogenic fungi were also detected from both groups of fields – *Fusarium oxysporum*, *Verticillium dahlia*, *Phoma eupyrena*.

4. The plant health, in terms of plant disease suppressiveness, had not improved – crop plants in organic agriculture fields were more severely attacked by *Phytophthora infestans*, and at the same level for *Streptomyces scabies* and *Rhizoctonia solani*.

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References

Bailey K. L., Lazarovits G. Suppressing soil-borne diseases with residue management and organic amendments // Soil and Tillage Research. – 2003, vol. 72, iss. 2, p. 169–180

Barnett H. L. Illustrated genera of imperfect fungi. – Minneapolis, USA, 1957, 218 p.

Biederbeck V. O., Zentner R. P., Campbell C. A. Soil microbial populations and activities as influenced by legume green fallow in a semiarid climate // Soil Biology and Biochemistry. – 2005, vol. 37, iss. 10, p. 1775–1784

Bloem J., de Ruiter P. C., Koopman G. J. et al. Microbial numbers and activity in dried and rewetted arable soil under integrated and conventional management // Soil Biology and Biochemistry. – 1992, vol. 24, iss. 7, p. 655–665

Bridge P., Spooner B. Soil fungi: diversity and detection // Plant and Soil. – 2001, vol. 232, iss. 1–2, p. 147–154

Chabrerie O., Laval K., Puget P. et al. Relationship between plant and soil microbial communities along a successional gradient in a chalk grassland in north-western France // Applied Soil Ecology. – 2003, vol. 24, iss. 1, p. 43–56

Choiseul J., Allen L., Carnegie S. F. Fungi causing dry tuber rots of seed potatoes in storage in Scotland // Potato Research. – 2007, vol. 49, iss. 4, p. 241–253

Cordier C., Edel-Hermann V., Martin-Laurent F. et al. SCAR-based real time PCR to identify a biocontrol strain (T1) of *Trichoderma atroviride* and study its population dynamics in soils // Journal of Microbiological Methods. – 2006, vol. 68, iss. 1, p. 60–68

Doneche B., Seguin G., Ribereau-Gayon P. Mancozeb effect on soil microorganisms and its degradation in soils // Soil Science. – 1983, vol. 135, iss. 6, p. 361–366

Drenth A., Janssen E. M., Govers F. Formation and survival of oospores of *Phythophthora infestans* under natural conditions // Plant Pathology. – 1995, vol. 44, iss. 1, p. 86–94

Drinkwater L. E., Letourneau D. K., Workneh F. et al. Fundamental differences between conventional and organic tomato agroecosystems in California // Ecological Applications. – 1995, vol. 5, iss. 4, p. 1098–1112

Elmholt S., Labouriau R. Fungi in Danish soils under organic and conventional farming // Agriculture, Ecosystems and Environment. – 2005, vol. 107, iss. 1, p. 65–73
Gabor E. M., de Vries E. J., Janssen D. B. Efficient recovery of environmental DNA for expression cloning by indirect extraction methods // FEMS Microbiology Ecology. – 2003, vol. 44, iss. 2, p. 153–163
Galván G. A., Parádi I., Burger K. et al. Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands // Mycorrhiza. – 2009, vol. 19, iss. 5, p. 317–328
Garbeva P., van Veen J. A., van Elsas J. D. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness // Annual Review of Phytopathology. – 2004, vol. 42, iss.29, p. 243–270
Gardes M., Bruns T. D. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts // Molecular Ecology. – 1993, vol. 2, iss. 2, p. 113–118
Gyaneshwar P., Naresh Kumar G., Parekh L. J., Poole P. S. Role of soil microorganisms in improving P nutrition of plants // Plant and Soil. – 2002, vol. 245, iss. 1, p. 83–93
Hagn A., Pritsch K., Schloter M., Munch J. C. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. // Biology and Fertility of Soils. – 2003, vol. 38, iss. 4, p. 236–244
Hagn A., Wallisch S., Radl V. et al. A new cultivation independent approach to detect and monitor common *Trichoderma* species in soils // Journal of Microbiological Methods. – 2007, vol. 69, iss. 1, p. 86–92
Hršelová H., Chvátalová I., Vosátka M. et al. Correlation of abundance of arbuscular mycorrhizal fungi, bacteria and saprophytic microfungi with soil carbon, nitrogen and phosphorus // Folia Microbiologica. – 1999, vol. 44, iss.6, p. 683–687
Huang P. M., Wang M. K., Chiu C. Y. Soil mineral-organic matter-microbe interactions: impacts on biogeochemical processes and biodiversity in soils // Pedobiologia. – 2005, vol. 49, iss. 6, p. 609–635
Jamiołkowska A., Wagner A. Fungal communities from the rhizosphere of tomato cultivated conventionally and with rye as cover crop // Electronic Journal of Polish Agricultural Universities. – 2005, vol. 8, iss. 4, p. 23
Kapsa J. S. Important threats in potato production and integrated pathogen/pest management // Potato Research. – 2008, vol. 51, iss. 3–4, p. 385–401
Kiffer E., Morelet M. The deuteromycetes. Mitosporic fungi. Classification and generic keys. – Enfield, UK, 2000, 273 p.
Liu B., Tu C., Hu S. et al. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight // Applied Soil Ecology. – 2007, vol. 37, iss. 3, p. 202–214
Lynch J. M., Benedetti A., Insam H. et al. Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms // Biology and Fertility of Soils. – 2004, vol. 40, iss. 6, p. 363–385
Marschner P., Kandeler E., Marschner B. Structure and function of the soil microbial community in a long-term fertilizer experiment // Soil Biology and Biochemistry. – 2003, vol. 35, iss. 3, p. 453–461
O'Donnell K., Gigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous // Molecular Phylogenetics and Evolution. – 1997, vol. 7, iss. 1, p. 103–116
Postma J., Schilder M. T., Bloem J., van Leeuwen-Haagsma W. K. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems // Soil Biology and Biochemistry. – 2008, vol. 40, iss. 9, p. 2394–2405
Shannon D., Sen A. M., Johnson D. B. A comparative study of the microbiology of soils managed under organic and conventional regimes // Soil Use and Management. – 2002, vol. 18, iss. 1, p. 274–283
Torsvik V., Sorheim R., Goksoyr J. Total bacterial diversity in soil and sediment communities – a review // Journal of Industrial Microbiology and Biotechnology. – 1996, vol. 17, iss. 3–4, p. 170–178
Treikale O., Priekule I., Pugačova J., Lazareva L. Occurrence of the *Fusarium* species and the risk of mycotoxins associated with head blight in winter wheat in Latvia (summary) // Latvian Journal of Agronomy. – 2008, vol. 10, p. 197–201 (in Latvian)

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Ekologinės žemdirbystės taikymo (šešerius metus) įtaka dirvos mikroorganizmams ir augalų ligų slopinimui

L. Grantina[1], K. Kenigsvalde[1], D. Eze[1], Z. Petrina[1], I. Skrabule[2], N. Rostoks[1], V. Nikolajeva[1]

[1]Latvijos universitetas
[2]Priekulės valstybinis augalų selekcijos institutas, Latvija

Santrauka

Tyrimų tikslas – kompleksiškai ištyrinėti dirvožemio mikrobų įvairovę ir nustatyti ekologinės žemdirbystės taikymo šešerius metus įtaką mikrobų bendrijoms po ilgalaikio tradicinės žemdirbystės taikymo. Analizuojant dirvožemio kultivuojamus mikroorganizmus taikyti klasikiniai mikrobiologiniai metodai, tiriant nekultivuojamus organizmus – molekulinės genetikos metodai. Per dvejų metų laikotarpį nustatyti ryškūs tyrinėtų kultivuojamų mikroorganizmų grupių bendro kiekio svyravimai tam tikruose laukuose tam tikrų pavyzdžių ėmimo metu. Visų analizuotų kultivuojamų mikroorganizmų grupių gerokai didesnis vidutinis kiekis buvo nustatytas ekologinės žemdirbystės laukuose, palyginti su tradicinės žemdirbystės laukais, pvz., bakterijų kiekis padidėjo 70 %, aktinobakterijų – 290 %, kultivuojamų siūlinių grybų – 110 %, mieles ir maltozę fermentuojančių bakterijų – 190 %. Grybų įvairovės ir *Trichoderma* spp. DNR kiekio tyrimų rezultatai, gauti taikant molekulinius metodus, tokio padidėjimo neparodė. Kitaip nei dirvožemio mikrobiologiniai rodikliai, augalų sveikata ligų slopinimo atžvilgiu nepagerėjo – ekologinės žemdirbystės laukuose augalai buvo labiau arba vienodai pažeisti patogeninių grybų ir bakterijų.

Reikšminiai žodžiai: ekologinė ir tradicinė žemdirbystė, mikrobų įvairovė, ARDRA, qPCR, ligų slopinimas.