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The status of soil organic matter decomposing microbiota in afforested and abandoned arable *Arenosols*

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Abstract

Organic matter decomposing soil microbiota status (abundance and diversity) have been investigated in former arable *Arenosols* (*AR*) that (i) were afforested 45 years ago with Scots pine (*Pinus sylvestris* L.) plantations or (ii) abandoned for the last 12 years (since 1993). The abundance of soil microbiota in soil organic layer (forest floor or dead grass fall) and in surface 0–2 and 2–10 cm mineral layers of former ploughed Ap horizon was estimated by the standard method of sowing organic or mineral soil suspensions on the agarised nutrient standard media and on media supplemented by mineral substances and different lignin monomers. The diversity of soil microbiota was determined only in surface mineral layers and was expressed according to the occurrence of the cytochrome P450 gene in mineral soil. The highest abundance of soil microbiota was found in organic layers of former arable *Arenosols*, especially, in forest floor of pine plantations, where mean abundance of microbiota was from 4–10 to even 20–30-fold higher than in surface mineral layers of former arable horizon. However, in the studied surface mineral soil layers mean microbial abundance, including lignin decomposing microbiota, was on average by 2–5 times higher in abandoned land than in pine plantations. In addition, it was determined that the microbiota diversity was two times higher in abandoned *Arenosols* where more actinobacteria and proteobacteria strains were, while in 45-year-old pine plantations organic matter decomposers were mainly proteobacteria strain related.
It was concluded that the accumulation and the decomposition of forest floor, and lower abundance and diversity of microbiota in surface mineral layer preserve or even could promote higher sequestration rate of soil organic carbon in afforested than in abandoned arable *Arenosols*.

Key words: abundance and diversity, afforestation or abandonment, arable *Arenosols*, lignin monomers, organic carbon, soil microbiota.

Introduction

Forests accumulate large quantities of carbon (C) not only in the biomass of trees and others forest plants but in forest soils as well. Median soil organic carbon (SOC) pool in the mineral soil of European forests is approximately 1.5 times as high as in the trees (De Vries et al., 2006). Meanwhile, the intensive cultivation of agricultural land and the removal of organic carbon (OC) with crop yield could cause a 30–50% loss of SOC (Virto et al., 2012). Therefore without the compensation of organic matter, like in practice of organic-sustainable farming, the SOC accumulation usually decreases in conventional-chemical farming systems (Fornara, Tilman, 2008; Bakšienė et al., 2014).

The European Parliament Directive on Soil Protection and Improvement (COM (2006) 232) emphasizes that SOC accumulation with the litter biomass is the key for soil conservation in the sustainable development of agriculture and forestry. Thus, along the carbon accumulation the forests may reduce CO_2 concentrations in the atmosphere (Dijkstra et al., 2009).

The natural or artificial afforestation is mostly common in unsuitable for agriculture or in abandoned former arable land. Such afforestation of inappropriate farming and unused state land could be relevant with the focus on carbon sequestration. This is especially important for the European Union countries committed to reduce CO_2 emissions following the Kyoto Protocol and reducing the negative effects of the climate change (Feller, Bernoux, 2008).

The afforestation of arable land, especially with coniferous trees, induces chemical changes in mineral topsoil. The accumulation and decomposition of the

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forest litterfall is influencing the destruction of former ploughed Ap horizon by the leaching of organic C acids and nutrients (mainly exchangeable cations of potassium, calcium and magnesium) that is indicated by increasing of mineral soil acidity (Lal, 2009). Due to the formation of a thin humus horizon in the surface of mineral soil, carbon stock increases in the surface 0–5 cm mineral soil layer but decreases in the deeper (5–25 cm) layer. Therefore, the initial carbon pools in 20–30 cm thick mineral topsoil tend to decrease during the first 5–10 years following afforestation of arable land. However, in forest plantations older than 30–40 years, the store of soil carbon has recovered to the original level and starts to increase (Bárcena et al., 2014; Malý et al., 2014). Such an increase is especially considerable in the nutrient-poor sandy soils with a low carbon stock (Armolaitis et al., 2007; Xiong et al., 2014).

The accumulation of SOC significantly depends on the abundance and activity of soil microbiota (Chaudhry et al., 2012; Tkacz et al., 2015). Only a part of OC is stabilized in soil humus or in the soil microbiota biomass. Even though, some studies have emphasized that, as an example, the sandy soils in abandoned agricultural land due to more intensive distribution of precipitation, higher soil temperature, better aeration and lower acidity contribute to a large and more active soil microbiota community if to compare with forest land (Lagomarsino et al., 2012; Müller-Stöver et al., 2012).

In this study we compared the soil microbiota status in nutrient-poor arable *Arenosols* (i) following afforestation with Scots pine and (ii) following abandonment of fertilized arable soils. Particularly, we focused on valuation of the abundance and diversity of soil microbiota that decompose OC compounds. We hypothesized that slowly degradable lignified organic matter could induce the reduction of soil microbiota abundance. Consequently, higher abundance and diversity of soil microbiota could decrease the sequestration of SOC.

Materials and methods

Study site. The study was carried out at 2005 in Perloja experiment (hereafter – Perloja experiment) of Perloja Experimental Station of Lithuanian Research Centre for Agriculture and Forestry. The Perloja experiment site (total area 40 ha) is situated in a flat area (elevation 110 m) in Southern Lithuania (54°10′ N, 24°25′ E). The mean annual precipitation of the area is 682 mm, whereas mean annual temperature is 6.2°C, with a mean January temperature of −5.4°C and a mean July temperature of 18.6°C. The soil in Perloja experiment site is well-drained *Haplic Arenosol* (*ARh*) (WRB, 2014) developed on glaciofluvial sandy deposits from Weichselian glaciation.

In 1960, a long-term Perloja experiment was established in arable land with the aim of comparing the productivity of arable land and arable land afforested with Scots pine (*Pinus sylvestris* L.). Details on the plots and treatments in the Perloja experiment were provided in Armolaitis et al. (2007). For our study we selected two sites of Perloja experiment: (i) non-fertilized 45-year-old Scots pine plantations and (ii) abandoned former fertilized arable land not cultivated for the last 12 years (since 1993).

The main chemical parameters of *Haplic Arenosol* in Scots pine plantations and adjacent abandoned former arable land of the Perloja experiment are presented in Table 1. In summary, first of all it could be stated that soil organic horizons as well as surface 0–2 and 2–10 cm mineral layers of former ploughed Ap horizon were of higher acidity (pH_{CaCl2} 3.6–4.7) in Scots pine plantations than in former arable land (pH_{CaCl2} 5.0–6.0). Also it was found that significantly higher content of organic carbon (OC) was accumulated in soil organic horizons (as forest floor) of pine plantations. Besides, under the forest floor in thin 0–2 cm deep mineral soil layer, the concentrations of soil organic carbon (SOC) and total nitrogen (N) were on average about 2–3 times higher in pine plantations than in abandoned former arable land. However, mean concentrations of mobile P_2O_5 and K_2O were still higher in surface mineral layers of abandoned formerly fertilized arable land.

Soil sampling and microbiota abundance. The following horizons of organic (O) layer of *Arenosol* were found and sampled (Cools, De Vos, 2016): OL – litter horizon (containing unaltered annual fall of needles, twigs and small branches, cones and bark in pine plantations and dead grass fall in abandoned arable land), OF – fragmented and/or altered horizon (containing partly decompose forest litter and only the remains of the autumn of the previous year grass cover in abandoned arable land) and OH – humus horizon (well-decomposed, dark and amorphous organic matter in pine plantations; it was absent in abandoned arable land). These horizons comprised forest floor in pine plantations while OF horizon – grass fall in abandoned land. Composite soil samples (n = 3) for analyses of soil microbiota abundance were collected from the OL and OF + OH horizons and from 0–2 and 2–10 cm deep surface layers of former ploughed Ap horizon in each plot at 10 systematically distributed points along the 20 meters transects. The organic layer was sampled using a 1000 cm^2 metallic

Table 1. Mean parameters of *Arenosol* in Scots pine plantations and adjacent abandoned former arable land of the Perloja experiment (adopted from Armolaitis et al., 2007)

Horizon / depth	Biotope	pH_{CaCl_2}	SOC g kg^{-1}	N g kg^{-1}	P_2O_5 mg kg^{-1}	K_2O mg g^{-1}
OL	plantations	3.7 ± 0.2	**545.7 ± 6.0**	9.4 ± 1.1	325 ± 37	584 ± 49
	arable land	**5.6 ± 0.2**	360.6 ± 17.2	10.9 ± 0.7	**650 ± 30**	**2593 ± 101**
OF + OH	plantations	3.6 ± 0.2	**386.7 ± 26.0**	8.1 ± 1.7	247 ± 6	384 ± 5
	arable land	**5.0 ± 0.1**	252.5 ± 16.5	9.3 ± 0.4	**660 ± 35**	**2815 ± 142**
Ap (0–2 cm)	plantations	3.6 ± 0.2	**28.7 ± 1.6**	**2.3 ± 0.2**	58 ± 8	146 ± 18
	arable land	**5.5 ± 0.2**	8.4 ± 1.0	1.3 ± 0.1	**136 ± 8**	179 ± 4
Ap (2–10 cm)	plantations	4.7 ± 0.4	**6.5 ± 0.3**	0.5 ± 0.1	50 ± 5	51 ± 2
	arable land	**6.0 ± 0.2**	5.7 ± 0.3	**1.0 ± 0.1**	**148 ± 11**	**114 ± 4**

Note. OL – litter horizon of organic layer, OF + OH – fragmented + humus horizons of organic layer (OH horizon was not found in abandoned arable land), Ap – former ploughed mineral horizon; means (n = 3) ± standard errors are given; significantly ($p < 0.05$) outstanding parameters are shown in bold.

circular frame while mineral soil – using metallic auger of 3 cm diameter.

The abundance of cultivable microbiota was distinguished by the standard method of the sowing of organic or mineral soil suspension on the agarised nutrient medium (Fierer et al., 2007). Although, the sowing of diluted gradually by 10, 100 and 1000 times soil solutions was performed using standard media and in addition the modified agarised nutrient media were applied. In total 9 media were used (Table 2). Nutrient media numbered by 5–9 were supplemented with the lignin monomers and the differentiation of lignin decomposing microbiota was performed due to the catabolic responses to the specific lignin monomers.

Table 2. Characteristics of the media for the estimation of microbial abundance in *Arenosol* in afforested with Scots pine plantations and abandoned arable of Perloja experiment

Medium number	Medium	Adaptability
	I. Standard medium	
1.	Plate-count agar / standard methods agar[1]	Total abundance of colony forming microbiota (bacteria, actinobacteria and micromycetes)
2.	Bacteriological agar (BA)[2]	Total abundance of colony forming bacteria
	II. Standard medium of bacteriological agar, supplemented by different mineral substances	
3.	BA and mineral soil solution extract[3]	Total abundance of colony forming microbiota assimilating soil mineral nutrients
4.	BA and mineral medium (MM, components of mineral medium: KH_2PO_4 – 2.3 g, $Na_2HPO_4 \times 12H_2O$ – 5.8 mg, NH_4Cl – 1.0 g, $NaHCO_3$ – 0.5 mg, $MgSO_4 \times 7H_2O$ – 2.000 ml, $CaCl_2 \times 2H_2O$ – 0.068 ml, $FeCl_3 \times 6H_2O$ – 0.511 ml, $C_6H_5O_7Na_3 \times 2H_2O$ – 0.221 ml; components adjusted to 1000 mL of solution with distilled water)[2]	Total abundance of colony forming microbiota assimilating mineral enrichments in medium
	III. Standard medium of bacteriological agar, supplemented by mineral substances and lignin monomers	
5.	BA and MM with 0.1% m-coumaric acid	Total abundance of colony forming microbiota assimilating specific carbon compounds (different lignin monomers)
6.	BA and MM with 0.1% cinnamyl alcohol	
7.	BA and MM with 0.1% caffeic acid	
8.	BA and MM with 0.1% cinnamic acid	
9.	BA and MM with 0.1% p-coumaric acid	

Note. Nutrient media were prepared according to: [1]Ikeda et al. (2009), [2]Anderson et al. (2011) and [3]Chaudhry et al. (2012).

Microbiota plates were incubated at 27°C. The morphology of the bacteria, actinobacteria and micromycetes colonies was assessed on plates after 5–7 days by the needle strike and the optical microscopy. Then the count of colony forming units (CFU) of the microbiota was estimated and the abundance of microbiota was evaluated for 1 g of to the constant dry (in 90°C) weight of the soil (Ikeda et al., 2009; van Elsas, Boersma, 2011).

Sampling and microbiota diversity. For analyses of soil microbiota diversity soil samples were collected in Scots pine plantations and abandoned arable land only from 0–10 cm deep mineral topsoil of the former ploughed Ap horizon. Homogenized soil samples were transferred to an Eppendorf tubes and frozen (at −80°C) and, thus, the bacteria diversity analyses were done in the Department of Molecular Microbiology and Biotechnology of Vilnius University, Lithuania. Diversity of soil microbiota in the Perloja experiment was estimated according to the sequences of the gene for the cytochrome P450 monooxygenases (hereafter – the cytP450) in the microbiota. The cytP450 are widely distributed in different life forms including prokaryotes (archea and bacteria), lower eukaryotes (fungi and insects) and higher eukaryotes (plants and animals) (Jung et al., 2011). The cytP450 constitute a large superfamily of heme proteins, performing the electron and proton transportations across the cell walls. The main function of the cytP450 is the inducing of the organic substrate degradation through the activation of the oxygen O_2 molecule, then the banding one O atom to the molecule of the organic substrate, and the banding of second O atom with the hydrogen consequently forming water molecule.

The extraction of bacterial DNA from soil was performed according to the instructions described by Reeve et al. (2010), using 1 g of the soil from each sample for the enzymatic and chemical destruction of soil sample. The DNA was then purified and precipitated for clear DNA pellets according to the instructions described by Bertrand et al. (2005). The bacterial 16S rRNA gene for cytP450 was amplified with two bacterial 16S rRNA primers OxyALK1FR 5'-GTG GGC GGC AAC GAC ACS CAN CGG AA-3' and OxyALK2R 5'-GCA CCG GTG GAT R(G/A)CC RAA CCC AAA3' ("Roth and Metabion", Germany; "Fermentas", Lithuania). The PCR machine Mastercycler Epgradient S PGR ("Eppendorf", Germany) was used for this amplification. The amplification was performed according to the manufacturer's instructions, using Taq-DNA polymerase and primers. The following conditions were applied in the amplification of the 16S rRNR genes: 94°C for 3 min followed by 29 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and final 10 min extension at 72°C.

The amplicons with 350 bp were analysed by electrophoresis on agarose gel with ethidium bromide staining and were evaluated from the gel by adding molecular gene ruler Gene Ruler™ DNA Ladder Mix ("Fermentas"), according to the manufacturer's instructions. The 16S rRNA gene amplicons obtained were inserted in vector pTZ57R/T, Inst/Aclone™ PCR Product Cloning Kit ("Fermentas"), following the manufacturer's instructions, and were applied for the transformation of *Escherichia coli* DH5α lines. After cultivating the transformed *E. coli* DH5α clones, plasmids were isolated according to the instructions described by van Elsas and Boersma (2011).

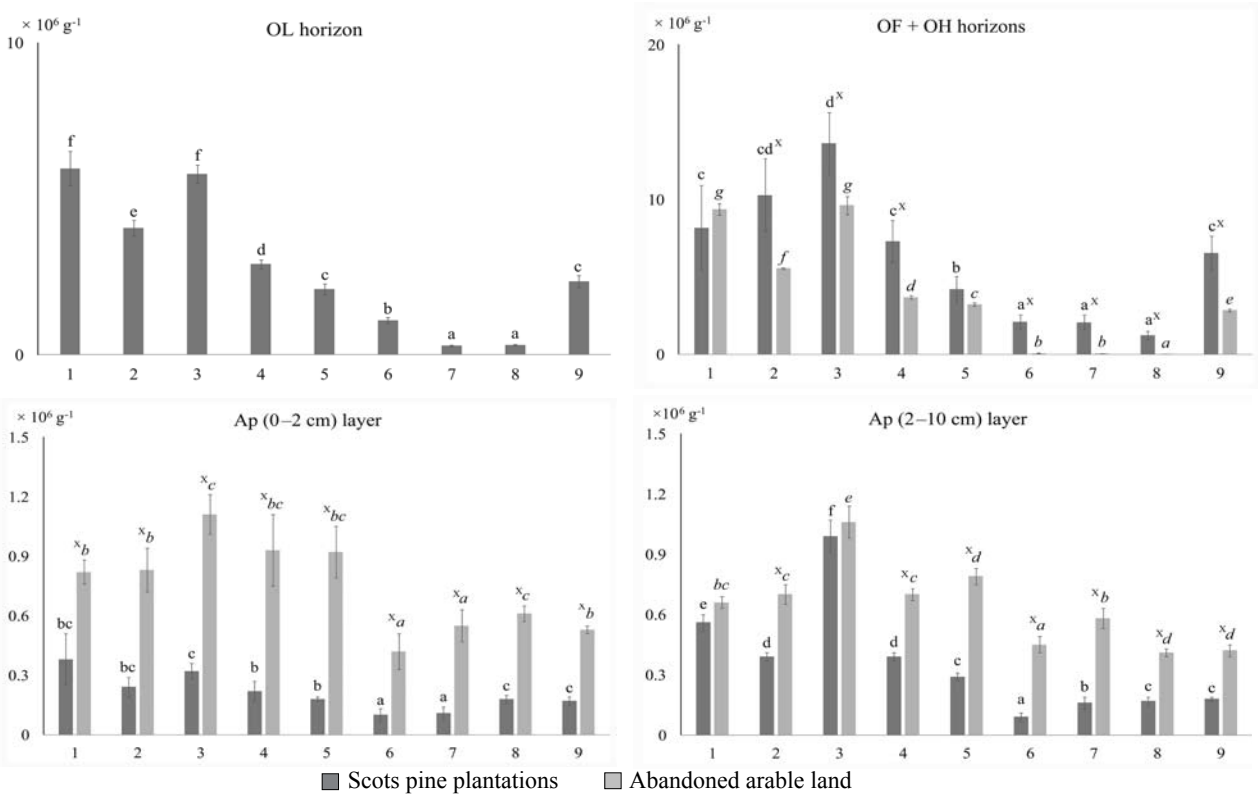
Determination and comparison of bacterial DNA aminoacid and nucleotide sequences. The 16S rRNR fragments inserted into the plasmid were digested with AscI, EcoRI, HindIII, EheI, PstI, BamHI, Bsp68I, SalI, KpnI, XhoI, SmaI, NcoI, NdeI, XbaI, AscI, EheI, Eco47III, Eco147I, Ecl136II and PaeI ("Fermentas"), following the manufacturer's instructions. The reaction products were separated with electrophoresis on agarose gel with ethidium bromide staining. Then 16S rRNA fragments were digested using the Perfectprep® Plasmid Mini set ("Eppendorf") and the oligonucleotide primers: M13/pUC(F)5'–GCCAGGGTTTTCCCAGTCACGA-3' and M13/pUC(R)5'-GAGCGGATAACAATTTCACACAGG-3', following the manufacturer's instructions ("Fermentas"). Representative clones showing unique cytP450 fragment length patterns were selected and sequenced in the Center of Sequencing Department of Molecular Microbiology and Biotechnology of Vilnius University, Lithuania. The nucleotide and amino acid sequences were compared directly to all known sequences deposited in GenBank databases using the basic local alignment search tool (NCBI BLAST). The parsimony phylogenetic trees were constructed by the Neighbour-Joining algorithm, with the model of nucleotide replacement using software *MEGA 3.1* with 1.000 bootstrap replicate.

Statistical analysis. The data was structured and Analysed using the softwares *Microsoft Excel 2010* and *STATISTICA 7.0*. Differences in the contrasting treatments were statistically evaluated by analysis of variance (*ANOVA*). In the case of significant effects, the direction of change and test of significance between treatment levels were estimated by pairwise *t*-tests. Significant differences in this study are a confidence at the level of $p < 0.05$.

Results and discussion

Abundance of soil microbiota. In the Perloja experiment, the highest mean total abundance of microbiota (bacteria, actinobacteria and micromycetes) was estimated in soil organic (OL and OF + OH) horizons of abandoned arable land and Scots pine plantations (Fig.). The microbiota in Scots pine forest floor was ranging from 0.29 to 5.79 million g^{-1} CFU (× 10^6 g^{-1} CFU). However, the abundance of microbiota in OF + OH horizons was more expressed. There the mean total microbiota comprised from 1.23 × 10^6 g^{-1} to 10.29 × 10^6 g^{-1} CFU and from 0.02 × 10^6 g^{-1} to 9.62 × 10^6 g^{-1} CFU, respectively, in afforested and abandoned land. In some cases (on 2 and 4 as well as on 6–9 nutrient media), in the OF + OH horizons the mean total abundance of cultivable microbota of abandoned arable land was significantly ($p < 0.05$) more than 1.8 times lower than in Scots pine plantations.

The mean total abundance of cultivable microbiota in mineral soil surface layers (up to 0–10 cm in depth) in abandoned arable land and Scots pine plantations was, respectively, on an average from 4 to 26 times and from 4 to 43 times lower than in soil organic horizons (Fig.). It was estimated that mean abundance of microbiota ranged from 0.45 × 10^6 g^{-1} to 1.11 × 10^6 g^{-1} CFU (in abandoned arable land) and from 0.08 × 10^6 g^{-1} to 0.99 × 10^6 g^{-1} CFU (in Scots pine plantations)



Notes. Means (n = 9) and standard errors (bars) of soil cultivable microbiota abundance evaluated on 1–9 – nutrient media (see Table 2). Asterisks denote significant differences between data obtained in Scots pine plantations and abandoned former arable land (^{X} – $p < 0.05$). Lower case letters (in *Italic* for abandoned arable land) denote the significant ($p < 0.05$) differences between nutrient mediums in particular biotope.

Figure. Mean total abundance of microbiota (× 10^6 g^{-1} CFU) in soil organic horizons and in upper mineral layers of *Arenosols* in Scots pine plantations and abandoned arable land of the Perloja experiment

in 0–2 and 2–10 cm mineral soil layers. However, the abundance of cultivable soil microbiota up to 0–10 cm in depth of mineral soil in abandoned arable land was on average about 2–5 fold significantly ($p < 0.05$) higher than in Scots pine plantations.

The higher content of easily degradable organic components in organic substrates determines the higher abundance of soil microbiota; meanwhile more lignified organic matter is slowly degradable (Huang et al., 2010). In contrast with OL horizons, where the lignified compounds composition is on average 23–50%, the decomposition of grass litters is faster as the lignified compounds in the biomass compose on average 2–18% (Kramer et al., 2013). In Perloja experiment, the abundance of lignin decomposing microbiota (on 6–9 nutrient media) in soil organic horizons ranged on average 0.1–4.45 × 10^6 g^{-1} and 1.2–10.1 × 10^6 g^{-1} CFU, respectively, in abandoned arable land and in Scots pine plantations (Table 2). It is supposed that from 2 to 12 times higher abundance of the microbiota in soil organic horizons could be influenced by the higher accumulation of the lignified compounds in forest floor of Scots pine plantations.

Nevertheless, in mineral soil layers the abundance of microbiota is increasing due to the increase in the content of more available organic carbon (Miltner et al., 2012). Consequently, in Perloja experiment mean abundance of lignin decomposing microbiota in mineral soil layer (up to 0–10 cm in depth) was about 4 and even 13 fold lower than in organic horizons, respectively, in abandoned arable land and in Scots pine plantations. Even the accumulation of organic carbon in mineral surface layers of the nutrient-poor *Arenosol* was higher in pine plantations (Table 1), the abundance of lignin decomposing microbiota was on average three fold higher in abandoned land than in Scots pine plantations.

The data on the abundance of soil microbiota have confirmed that due to the complexity of organic material accumulated in the soils, the increase in the abundance of soil microbiota was estimated (Lagomarsino et al., 2012; Trujillo-Cabrera et al., 2013). Thus, the accumulation of forest floor in afforested *Arenosol* could determine in about two times higher abundance of organic soil microbiota in soil organic horizons of Scots pine plantations than in grass fall of abandoned arable land of Perloja experiment. However, from about 2 to about 5 fold higher abundance of soil microbiota was estimated in mineral soil surface layers of abandoned arable land. As it was mentioned, the higher abundance of microbiota in the abandoned land could have been determined by lower soil acidity, more intense precipitation, and beneficent soil aeration and higher soil temperature (Müller-Stöver et al., 2012; Alluvione et al., 2013).

Taxonomic diversity of soil microbiota. In the microbiota taxonomic classification, the cytochrome P450 monooxygenases are reflective in the differentiation of microbiota on the metabolic demand (Hlavica, 2013). While the microbiota cytP450 might be involved in the degradation of many aromatic compounds including lignin monomers, the prevalence of lignin decomposing bacteria could be estimated (Hungria et al., 2009; Huang et al., 2010). Our studies on soil taxonomic analysis coupled with the standard sequence homology criteria for the cytP450 revealed that the analyzed nucleotide sequences of the clones corresponding to the closest taxonomic homology in the GenBank significantly fell into the *Bradyrhizobium*, *Caulobacter*, *Rhodococcus* and *Rhodopseudomonas* species phyla (Table 3). According to the nucleotide sequences for the cytP450, in total 50 clones of microbiota were detected in Scots pine plantations and 113 clones in abandoned land.

Table 3. Distribution of the clone nucleotide sequences for the cytP450 from mineral topsoil in Scots pine plantations and abandoned arable land

	Gene or species / code in GeneBank[1]	Closest taxonomic neighbour[2]		Number of clones[3]	
		type / class	order / family	plantations	abandoned land
1.	*Bradyrhizobium japonicum* USDA 110/ NP 773883 put.cytP450	*Proteobacteria Alphaproteobacteria*	*Rhizobiales Bradyrhizobiaceae*	11	28
2.	*Caulobacter crescentus* CB 15/ YP 783213/ NP 418882 cytP450 family protein	*Proteobacteria Alphaproteobacteria*	*Caulobacterales Caulobacteraceae*	5	0
3.	*Rhodococcus* sp. DEE 5316/ DQ847175 cytP450 CYP153B7	*Actinobacteria Actinobacteria*	*Actinomycetales Nocardiaceae*	0	15
4.	*Rhodococcus* sp. MOP 100/ DQ847177 cytP450 CYP153B7	*Actinobacteria Actinobacteria*	*Actinomycetales Nocardiaceae*	0	15
5.	*Rhodopseudomonas palustris* BisA53/ CP000463 cytP450	*Proteobacteria Alphaproteobacteria*	*Rhizobiales Bradyrhizobiaceae*	29	53
6.	*Rhodopseudomonas palustris* Ha A2/ NC 007778 cytP450	*Proteobacteria Alphaproteobacteria*	*Rhizobiales Bradyrhizobiaceae*	1	0
7.	Uncultivated bacteria BAE 47482 cytP450 alkane hidroxilase	Uncultivated bacteria BAE 47482 cytP450 alkane hidroxilase	Uncultivated bacteria BAE 47482 cytP450 alkane hidroxilase	4	2
	Total number of clones			50	113

[1] – GenBank accession number of the nearest isolates, [2] – closest taxonomic homology in the GenBank, [3] – number of isolates belonging to each phylotype

The majority of the nucleotide sequences of clones from abandoned land displayed the higher similarity to *Bradyrhizobium* (28 clones), *Rhodococcus* (30 clones) and *Rhodopseudomonas* (53 clones) (Table 3). Then, the nucleotide sequences in Scots pine plantations – to *Bradyrhizobium* (11 clones), *Caulobacter* (5 clones) and *Rhodopseudomonas* (30 clones). In these subclasses the dominant species related to our studied clones of both studied biotopes are proteobacteria of the alpha subclasses. *Alphaproteobacteria*, *Rhodopseudomonas* and *Bradyrhizobium* grow in symbiosis with the plants. For example, *Rhodopseudomonas* bacteria are degrading biomacromolecules in soil and may fix the nitrogen and implement the denitrification processes (Anderson et al., 2011). *Bradyrhizobium* bacteria activate the root nodulation in plants for the atmospheric nitrogen fixation.

Many individuals of the *Alphaproteobacteria* are used in soil bioremediation due to their ability to degrade the polyaromatic hydrocarbons (Kleber, Johnson, 2010). Therefore, it is worth mentioning that the distribution of *Rhodopseudomonas* and *Bradyrhizobium* species were by 1.8 times higher in abandoned land than in Scots pine plantation (Table 3).

The cytP450 nucleotide sequences of five clones from mineral topsoil in Scots pine plantations were represented by species (*Caulobacter*) of the alphaproteobacteries (Table 3), that are distinguished as a worldwide abundant bacteria species in the soils as well as in open water reservoirs (Kleber, Johnson, 2010). It was estimated that *Caulobacter* is adapted to survive in the environments with a low concentration of nutrients (especially phosphorus). While *Caulobacter* may decompose different organic compounds, such as, sucrose, amino acids, fatty acids and alcohols, they are used in creation of the enzymes.

The cytP450 nucleotide sequences of the clones representing the *Rhodococcus* species of the order *Actinomycetales* are the prevalent organic matter decomposers in soils (Trujillo-Cabrera et al., 2013). *Actinomycetales* are able to synthesize cellulose and hemicellulose-degrading enzymes, though, to degrade lignin (Baldrian et al., 2011). In many cases, actinomycetes were found in soils that are rich in organic matter; their abundance in cultivated agricultural land depends directly on the soil fertility (Chaudhry et al., 2012). Moreover, the representatives of the uncultivated bacteria (BAE 47482 on the correspondence to cytP450 alkane hidroxilase) were estimated (Table 3). The majority of the uncultivated bacteria prevailed in abandoned arable soils of Perloja experiment. However, the involvement of uncultivated bacteria in the lignin decomposition demands could not yet be clearly explained. In addition, the importance of their involvement in biogeochemical lignin conversions and in the degradation of particular lignin compounds was indicated (Kleber, Johnson, 2010).

It could be concluded that soil microbiota of *Arenosols* in Scots pine plantations and abandoned arable land produced the gene for the cytP450, and there were more than 3000 clones with DNA for the cytP450 obtained. However, in total 199 randomly picked clones displayed convincing homology to the cytP450 genes, with 50 and 113 clones, respectively, in Scots pine plantations and in abandoned arable land. More than 80% of clones for the cytP450 showed the homology with *Bradyrhizobium*, *Caulobacter*, *Rhodococcus* and *Rhodopseudomonas* species phyla. Thus, the clones of abandoned arable land formed the trends of homology to actinobacteria and proteobacteria, while the clones of Scots pine plantations – only to proteobacteria. This could be caused by a loss of weakly competitive non-specialized species, to the benefit of highly competitive or narrow niche decomposition specification. According to that, in the Perloja experiment even the clones of abandoned arable land are more diverse, the soil microbiota clones of the Scots pine plantations in some extent show the maturity of the forest soils.

Soil microbiota and organic matter accumulation. In this study however, higher soil microbiota abundance in organic horizons in Scots pine plantations as well as in abandoned arable land was relative due to the diverse formation of studied soil organic horizons (Fig.). Though, soil microbiota also showed the higher specificities, in particular decomposition of lignified organic matter, which could be strongly affected by the afforestation (Cotrufo et al., 2013). However, lower diversity in bacteria communities could lead to an adjustment of the efficiency of carbon use. This was the distinguished outcome in Scots pine plantations and agrees with other studies of this issue (Bruun et al., 2015). In contrast, even though there is an extent in organic matter in abandoned arable land through the OF + OH horizons and abundance of bacteria due to less lignified components in organic matter, bacteria could accelerate organic carbon lost. However, soil microbiota abundance also responds to the soil conditions. According to the previous study of Armolaitis et al. (2007) in Perloja experiment the organic horizons in plantations were significantly more acid – by 1.4–1.9 pH_{CaCl2} units (Table 1) and contained higher concentrations of carbon. In this context, our study confirmed that the microbiota of soil organic horizons was more active not in pine plantations but in abandoned land (Fig.).

The evident increase in soil microbiota was observed in 0–2 cm mineral topsoil in abandoned arable land (Fig.). Moreover, it could be explained by organic substrate availability in abandoned arable land. In contrast, due to more chemical stabilization of organic compounds decomposition in former Ap horizon in pine plantations was more limited. These changes showed that availability of labile carbon source reduce microbial activity (Bouasria et al., 2012; Jangid et al., 2013). The same tendency was obtained by Armolaitis et al. (2007) in Perloja experiment. The significant changes of organic carbon in mineral soil were found in the uppermost 0–2 cm layer of the former Ap horizon, where carbon pools were on average more than three times greater in pine plantations (0.72 kg m^{-2} C) than in abandoned arable land (0.22 kg m^{-2} C). For that, the decrease in organic matter degradation could increase carbon accumulation, while an increase in degradability might result in the depletion of soil carbon (Talbot, Treseder, 2012).

The afforestation of nutrient-poor sandy soils could result in an increase of soil C stores (Fornara, Tilman, 2008; Vesterdal et al., 2012). As compared with the recently abandoned arable land, carbon pools were considerably greater in pine plantations, mainly because of the accumulation of soil organic horizons (forest floor). Meanwhile, despite the low decomposition rate of soil organic layers, a significant increase in carbon pools was found in a 0–2 cm surface mineral layer in pine plantations. The reason could be more active soil microbiota as it was also reported by Poeplau and Don (2013). On the other hand, the accumulation and low decomposition rate of forest organic layers result at least in the maintenance of carbon sequestration in the underlying mineral soil horizons. Thus, the preservation of organic carbon does not occur in abandoned formerly arable land.

Conclusion

Along with the afforestation of arable *Arenosol* with Scots pine, the plantations were accumulating forest floor (organic OL, OF and OH horizons), where high abundance of organic compounds decomposing microbiota was obtained. Meanwhile in abandoned arable land grass litter was on average 12 fold lower with twice lower abundance of cultivable microbiota. However, in mineral topsoil (up to 0–10 cm in depth) mean abundance of cultivable soil microbiota in abandoned land was by 2–5 fold higher than in Scots pine plantations. Even more, the microbiota diversity was two times higher in abandoned *Arenosol* as more actinobacteria and proteobacteria strains were estimated, while in pine

plantations organic matter decomposers were mainly proteobacteria strain related. Summarizing the obtained results, it could be emphasized that in afforested arable land the accumulation and decomposition of forest floor and lower abundance and diversity of microbiota preserve or even increase the sequestration rate of soil organic carbon in surface mineral layer to a higher extent than in abandoned arable *Arenosol*.

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Dirvožemio organines medžiagas skaidančios mikrobiotos būklė apželdintuose ir dirvonuojančiuose ariamuose smėlžemiuose

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Santrauka

Organines medžiagas skaidančios dirvožemio mikrobiotos ypatybės (gausumas ir įvairovė) tirtos artuose smėlžemiuose (SD), kuriuose (i) prieš 45 metus įveisti paprastosios pušies (*Pinus sylvestris* L.) želdiniai arba (ii) buvusios žemės ūkio naudmenos dirvonavo 12 metų. Dirvožemio mikrobiotos gausumas vertintas dviejų horizontų – organinių (miško paklotės bei dirvone susiformavusio žolyno apmirusių nuokritų) ir mineralinių (buvusiame artame Ap horizonte) – 0–2 bei 2–10 cm gylio smėlžemių sluoksnių suspensijų sėjos ant agarizuotų etaloninių ir papildytų mineralinėmis medžiagomis bei įvairiais lignino monometrais mitybinių terpių metodu. Mikrobiotos įvairovė smėlžemių viršutiniame mineraliniame 0–10 cm gylio sluoksnyje nustatyta pagal citochromo P450 geno atitikties dažnumą. Didžiausias dirvožemio mikrobiotos gausumas buvo nustatytas artų smėlžemių organiniuose horizontuose ir ypač pušies želdinių miško paklotėje, kur vidutinis mikrobiotos gausumas buvo nuo 4–10 iki net 20–30 kartų didesnis nei dirvožemio viršutiniuose mineraliniuose sluoksniuose. Tačiau dirvonuojančių artų smėlžemių viršutiniuose mineraliniuose sluoksniuose vidutinis mikrobiotos, taip pat ir ligniną skaidančios mikrobiotos, gausumas buvo vidutiniškai 2–5 kartus didesnis nei pušies želdiniuose. Be to, dirvonuojančiuose smėlžemiuose nustatyta du kartus didesnė mikrobiotos įvairovė, nes juose esanti mikrobiota buvo gimininga aktinobakterijoms ir proteobakterijoms, o 45 metų amžiaus pušies želdiniuose organines medžiagas skaidanti mikrobiota – tik proteobakterijoms.
Apibendrinus tyrimo rezultatus galima teigti, kad dėl miško paklotės kaupimosi bei skaidymosi ir mažesnio mikrobiotos gausumo bei įvairovės stabilizuota organinės anglies apytaka anglies sekvestravimą skatina daugiau mišku apželdintų ariamų dirvožemių viršutiniuose mineraliniuose sluoksniuose nei dirvonuojančiuose smėlžemiuose.

Reikšminiai žodžiai: apželdinta arba dirvonuojanti žemė, ariami smėlžemiai, dirvožemio mikrobiota, gausumas ir įvairovė, lignino monomerai, organinė anglis.