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# THE CONTINGENCY OF SOIL MICROORGANISMS AND THE SELECTED SOIL BIOTIC AND ABIOTIC PARAMETERS UNDER DIFFERENT LAND-USES

JANA JÚDOVÁ<sup>1</sup>\*, RADOSLAVA KANIANSKA<sup>2</sup>, JANA JAĎUĎOVÁ<sup>2</sup>, MIRIAM KIZEKO-VÁ<sup>3</sup>, JARMILA MAKOVNÍKOVÁ<sup>4</sup>

#### Abstract

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Land use changes are local phenomena with global impact. They have an impact in a cumulative sense on biodiversity or soil degradation. This study aimed to examine the effects of different land-uses (arable land, permanent grasslands, abandoned grasslands, forest land) on the selected biotic and abiotic soil parameters in the Slovak mountain study sites Liptovská Teplička and Tajov. Biotic (microbial community structure, earthworm number and fresh body biomass, arthropod number and fresh body biomass), and abiotic chemical soil parameters (pH, total organic carbon, total nitrogen, nutrients) were measured. According to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight), several bacterial strains were identified. Mutual relations between soil microorganisms and soil biotic and abiotic properties determined by different land uses were analysed. Microbial response expressed as average well-colour development (AWCD) values indicated relations between higher microbial diversity and higher nutrient availability at both study sites. In the comparison of land use types, permanent grasslands (PG) showed the lowest microbial activity in the depth of 0-0.1 m. But in the depth of 0.2-0.3 m in PG of both study sites, the higher microbial activity was recorded compared to the depth of 0-0.1 m. In addition, lower AWCD values in PG were in line with the lower available P and K content but higher earthworm density and biomass.

Key words: biotic soil properties, chemical soil properties, MALDI, arable land, permanent grasslands, abandoned grasslands, forest land.

### Introduction

Soils are heterogenous mixtures of mineral, organic and biological compounds, which are frequently associated in complex hierarchical structures. Microbes adapt to microhabitats

<sup>&</sup>lt;sup>1</sup>Faculty of Industrial Technologies, Trenčín University of A. Dubček, 020 01 Púchov, Slovak Republic; e-mail: jana. judova7@gmail.com

<sup>&</sup>lt;sup>2</sup>Faculty of Natural Science, Matej Bel University, 974 01 Banská Bystrica, Slovak Republic

³National Agricultural and Food Centre – Grassland and Mountain Agricultural Research Institute, 974 21 Banská Bystrica, Slovak Republic

<sup>&</sup>lt;sup>4</sup>National Agricultural and Food Centre - Soil Science and Conservation Research Institute Bratislava, Regional station Banská Bystrica, 974 04 Banská Bystrica, Slovak Republic

<sup>\*</sup>Author for correspondence

and live together in consortia interacting with each other and their environment. Microbial diversity is critical to ecosystem functioning due to diversity of process, such as decomposition, nutrient cycling, soil aggregation and pathogenicity (Dubey et al., 2006) According to a current estimate, 1 g of soil may harbour up to 10 billion bacteria of possibly 4000–7000 different species and a biomass density of 30–30,000 kg.ha<sup>-1</sup> (Rosello-Mora, Amann, 2001).

The microbial members of soil communities are the most sensitive and rapid indicators of perturbations and land use changes. Land use change is a key component of global changes and largely impacts ecosystem structures, processes and functioning (Don et al., 2011). Soil biota contributes substantially to the resistance and resilience of agroecosystems to abiotic disturbance and stress (Brussaard et al., 2007). But the excessive use of pesticides can drastically modify the function and structure of soil microbial communities (Pampulha, Oliveira, 2006). Soils subjected to disturbance by tillage, however, can be more susceptible to reductions in soil microbiota due to desiccation, mechanical destruction, soil compaction, reduce pore volume, and disruption of access to food resources (Giller, 1996).

Increase in land use intensity, due to the demands of bioeconomy (production of food, feed, fuel and fibre), is also frequently observed in grassland ecosystems. While in the past, sites have been used extensively as pastures, nowadays, up to four times per season, the same areas are managed as meadows for hay production and silage, entailing an intensive application of organic and inorganic fertilizers (Meyer et al., 2013). Traditional agricultural land-scape consists of a mosaic of small-scale arable fields and permanent agricultural cultivation such as grasslands. They are significant as unique islands of species-rich plant and animal communities (Barančoková, Barančok, 2015). Differences in intensity of agricultural practice like mowing, grazing and fertilization lead to changes in land use, plant composition (Kaschuk et al., 2009, Thurston et al., 1969, Kirkham et al., 1996), microclimate, soil quality, and hence, to changes on macro- as well as micro-scale habitats. For example, Patra et al. (2006) compared the diversity pattern of microbial community involved in nitrogen fixation, denitrification and nitrification in grassland ecosystems under different management intensities. This study clearly demonstrated the changes in the diversity pattern of single functional groups involved in nitrogen transformation on low diverse grassland sites.

Soil microorganisms, mainly fungi and bacteria, are primarily responsible for the transformation of organic molecules in soil, and their activity is thus a key factor in SOM dynamics (Coq et al., 2007). Soil microorganisms are the decomposers of litter and SOM (soil organic matter) in terrestrial ecosystems, which can regulate multiple input and loss pathways of soil C and nitrogen (N) (McGuire, Treseder, 2010, Smith et al., 2014). It has been suggested that land use change can affect the microbial decomposition of litter and SOM, which in turn regulates soil C and N balance in terrestrial ecosystems (Brackin et al., 2013, Bossio et al., 2006).

Soil biota has considerable direct and indirect effects on crop growth and quality, nutrient cycle quality and the sustainability of soil productivity (Roger-Estrade et al., 2010). The researchers evaluate not only the roles of total carbon and nitrogen on microbial diversity but also the role of other nutrients, their availabilities and ratios, together with land use, climate and biotic and abiotic factors. Delgado-Baquerizo et al. (2016) found that bacterial diversity and composition is primarily driven by variation in soil resources stoichiometry (total C:N:P

ratios), itself linked to different land uses, and secondarily driven by other important biodiversity drivers such as soil pH, root influence and so on.

The study reports the microbial activity and diversity under different land use types in the year 2015 (partially in 2014) in the Slovak mountain regions with the following objectives: (1) to determine microbial activity and diversity in two different soil types and four land use types; (2) to determine selected soil chemical properties in different soil types and land use types (3) to evaluate mutual relations between soil biotic and abiotic parameters.

### Material and methods

Study site characteristics

Slovakia is predominantly situated in the western Carpathian arch. Nowadays, agricultural land in Slovakia occupies almost 50% of the total area of Slovakia. But during the formation period of Neolithic agriculture, the Carpathians were almost completely covered with forests (Ložek, 1973). It means that the landscape of Slovakia has undergone significant changes in land-use and cover until now. The study was carried out in 2014–2015 on two study sites,

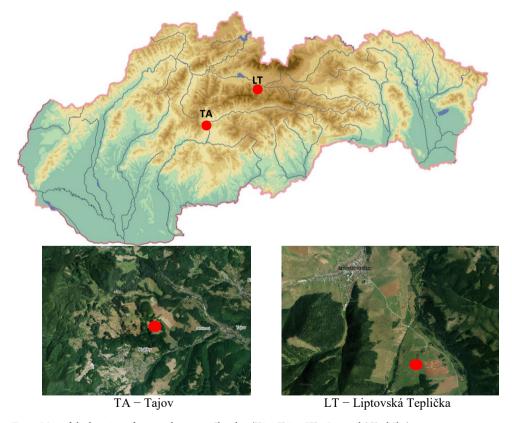


Fig. 1. Map of the location of two study sites in Slovakia (TA – Tajov, LT – Liptovská Teplička).

Rendzic Leptosol (FAO, 2014) developed on limestones in the region of north-eastern Slovakia at Liptovská Teplička (LT), and Haplic Cambisol (dystric variety) (FAO, 2014) developed on slope sediments in the region of central Slovakia at Tajov (TA). The LT area is situated in the Low Tatras Mountain at 950 m a.s.l. The long-term average annual air temperature is 6.2 °C, and long-term average annual rainfall is 950 mm. From the long-term point of view, the main land-use trend observed in LT was gradual afforestation and permanent grassland conversion to forest land (Kanianska et al., 2014). At present, extensive land abandonment in mountain rural areas continues. The TA area is situated in the Kremnica Mountain at 595 m a.s.l. The long-term average annual air temperature is 7.2 °C, and long-term average annual rainfall is 795 mm. The landscape suffers many changes that reflects in soil properties and mutual interactions between biotic and abiotic parameters. Therefore, we observed biotic with emphasis on soil microorganisms and abiotic properties and their mutual interactions in four different land uses in LT (arable land – AL, permanent grasslands – PG, abandoned grasslands – AG, forest land – FL), and in two different land uses in TA (permanent grasslands – PG, forest land – FL). In LT, the arable land is under organic farming, and the permanent grasslands are used as a meadow, and are mown for hay. In TA, the permanent grasslands are grazed by sheep.

### Biotic soil properties methods

The investigation was conducted in the autumn season in 2015; the selected analyses was conducted in the autumn and spring seasons in 2014. From the biotic soil properties, the microbial communities and their diversity were investigated.

Soil samples for microbial analyses were collected from 0–0.1 m and 0.20–0.30 m depth from four points placed at the apices of a 10 m side square at both study sites (LT, TA), and in the available land-use types (AL, PG, AG, FL). Soil samples from autumn 2015 were processed for community level physiological profiling (CLPP) using Biolog EcoPlates. Selected colonies from the samples of autumn 2015 and also spring and autumn 2014 were analysed according to Matrix Assisted Laser Desorption Ionization-Time of Flight Bruker Daltonik MALDI Biotyper, MicroflexLT (MALDI-TOF).

### Microorganism analysis - The Biolog EcoPlates

Microbial communities offer a potentially powerful forum for advancing the understanding on how community processes affect ecosystem processes. Also, active organisms, not culturable using solid agar, can respond in the CLPP. Organisms adapted for high nutrient concentration and rapid growth (like Pseudomonads) responds well on this assay (Garland, 1997). The Biolog EcoPlate (Garland, 1997) contains 31 of the most useful carbon sources for soil community analysis. Communities of organisms gave a characteristic reaction pattern called a metabolic fingerprint. Catabolism of each carbon substrate produced a proportional colour change response (from the colour of the inoculant to dark purple) due to the activity of the redox dye tetrazolium violet (present in all wells including blanks). The homogenized soil sample (1 g) was incubated in 99 ml of sterile water for 20 min. at 20 °C. Environmental samples' (150  $\mu$ l) suspensions (soil) were then inoculated directly into Biolog MicroPlates (Frac et al., 2012). The Biolog MicroPlates were incubated in the dark at 25 °C and analysed at defined time intervals over 2–5 days using microPlate reader by absorbance at 590 nm (Technical University, Zvolen, Slovakia).

The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changes from colourless into purple. The changes in the pattern were compared via the Principle Components Analysis (PCA) of the average well colour development (AWCD) data (Firestone et al., 1997; Cartwright, 2015). Microbial response in each microplate that expressed average well-colour development (AWCD) was determined as follows AWCD =  $\Sigma$  ODi/31 (Gomez et al., 2004), where ODi is optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source) values from each plate well. The changes observed in the fingerprint pattern provide useful data about the microbial population changes over time, and generate a microbial community summary variable based on substrate utilization.

Microorganism analysis - Cultivation and Matrix assisted laser desorption/ionization, time of flight - MALDI-TOF

Selected microbial colonies were analysed according to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight Bruker Daltonik MALDI Biotyper, MicroflexLT, Matej Bel University, Banska Bystrica, Slovakia). Preparation of soil samples' suspensions: 0.5 g of soil was homogenized in 2 ml of sterile phosphate buffer (PBS). Water and soil suspension samples /50–100 µl/ were placed on TSA (Trypton Soya agar, Oxoid) medium and culti-

vated for 24 hours by 35 °C, and then, the concentration of colonies, colony forming units (cfu) were done. Selected colonies were analysed according to the standard microbial protocols and according to the dry and semi-extraction procedure of MALDI-TOF (Matrix assisted laser desorption/ionization, time of flight) method (Bruker protocols).

### Soil chemical and physical analyses

The investigation was conducted in the autumn season in 2015. On each land-use type, soil samples for chemical analysis were collected from the same places like samples for microbial analyses, from four points placed at the apices of a 10-m side square. Soil samples were air dried, homogenized, sieved on 2 mm sieves and analysed according to the methods applied in Partial monitoring system – Soil (Fiala, 1999). Soil pH was determined in KCl, total oxidizable organic carbon (TOC) according to Tjurin's method in the modification of Nikitin, total nitrogen (N<sub>1</sub>) according to the modified Kjeldahl method in accordance with the Slovak standardized method (STN ISO 11261), available nutrients (P, K, Mg) according to Mehlich III. The average values were used as soil chemical status characteristics. Content of clay was determined by the pipette method according to Novak.

### Earthworm and arthropod sampling and determination

The investigation was conducted in the spring season in 2015. We used the obtained results for the evaluation of the possible mutual relations between microorganisms and soil meso (soil arthropods) and macrofauna (earthworms).

Earthworms and soil arthropods were sampled in each land-use type. At each site, earthworms were hand sorted from seven soil monoliths  $(0.35 \times 0.35 \times 0.20 \text{ m})$  placed in line in 3 m distance. The earthworms were counted and collected. Earthworms from deeper soil layers were expelled by 1.5 L of 0.2% formalin. The collected samples of earthworms were transported in glass cups with sufficient amount of soil in portable fridge to laboratory. The collected earthworms were washed, weighted and the body colour was noted. The earthworm density and biomass from soil monoliths were recalculated per square meter (Kanianska et al., 2016).

Soil arthropods were sampled from the same places where earthworms were collected into the 7 plastic traps placed flush with the surface of the soil in line in 3 m distance. The traps were filled with 200 ml formalin solution, which acted as a killing and preserving agent. After one month, the traps were collected and the material was weighed. The captured individuals were preserved in formalin solution, identified, and the total number of each one was recorded and classified in taxonomic categories (orders) (Jaďuďová et al., 2016). Quantitative composition was expressed as number of soil arthropod individuals (ind.trap¹) and fresh body biomass (g.trap¹).

### Results

## Microbial community structure

Measurements of metabolic traits through different carbon sources utilization were used like an indicator of biotic parameters in different soil communities. In the abandoned grasslands (AG) and forest land (FL) at Liptovská Teplička (LT) and in the forest land (FL) at Tajov (TA), more colour intensity differences were obtained than in the permanent grasslands (PG) and arable land (AL) with a lot of colourless results, especially at TA (Fig. 2).

We compared the average well-colour development data at the two study sites and different land-uses (Tables 6, 7 and 8). At LT, based on the AWCD data we can assume in the depth of 0–0.1 m, the highest microbial diversity in AG (0.820) followed by AL (0.685) and FL (0.698). The lowest microbial diversity was found in PG (0.290). Similarly at TA, the higher microbial diversity based on AWCD data can be assumed in FL (0.801) as compared to PG (0.286). In the depth of 0.2–0.3 m in PG at both study sites, the microbial activity was higher as compared to the depth of 0–0.1 m but still the lowest as compared to the other land uses. The microbial activity at LT in AL was in the depth of 0.2–0.3 m lower (0.433) than in the

depth of 0-0.1 m (0.685); in AG, the situation was opposite and the microbial activity was slightly higher in the depth of 0.2-0.3 m (0.877) than in the depth of 0-0.1 m (0.820).

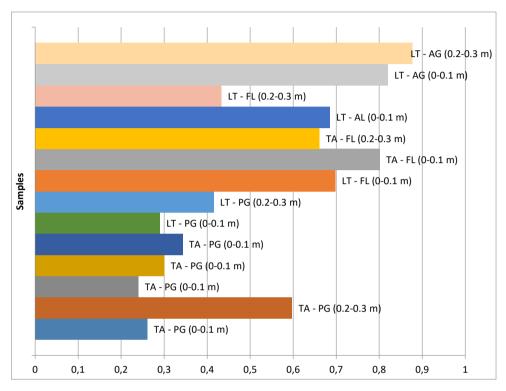


Fig. 2. Average well-colour development (AWCD) comparison of samples.

Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

# Microbial community diversity

Selected microbial colonies after cultivation of TSA (Trypton Soya Agar) were analysed according to MALDI-TOF at LT and TA study sites. Several bacteria strains were identified in different land uses and in different time period (Tables 1– 5).

In autumn samples from 2014 and 2015, several bacterial strains were obtained that were more variable in comparison with the spring samples strains, probably because of low temperature in the environment and slow start of biological processes in the soil.

There were found different strains of microorganisms like *Acinobacter calcoaceticus*, *Stenophomonas rhizophila*, *Solibacilus silvestris*, *Pseudomonas fluorescens*, *P. frederiksbergensis* and *Serratia proteamaculans*. At LT, the most frequent microorganisms in autumn 2015

T a b l e 1. MALDI-TOF results at Liptovská Teplička in autumn 2015.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(++)(A)	AL1 (0-0.1 m)	Pseudomonas frederiksbergensis	2.117	Pseudomonas brassicacearum	1.916
(+)(B)	AL1 (0.2-0.3 m)	Stenotrophomonas rhizophila	1.867	not reliable identification	1.434
(+)(B)	AL2 (0.2-0.3 m)	Acinetobacter calcoaceticus	<u>1.895</u>	not reliable identification	1.678
(+)(B)	PG1 (0-0.1 m)	Pseudomonas caricapapayae	<u>1.79</u>	not reliable identification	1.598
(+)(B)	PG1 (0.2-0.3 m)	Stenotrophomonas sp	1.992	not reliable identification	1.362
(+)(B)	PG2 (0.2-0.3 m)	m) Acinetobacter calcoaceticus		Acinetobacter calcoaceticus	1.821
(+)(B)	PG3 (0.2-0.3 m)	Acinetobacter calcoaceticus	1.872	Acinetobacter calcoaceticus	1.869
(++)(A)	AG1 (0-0.1 m)	Acinetobacter calcoaceticus	2.268	Acinetobacter calcoaceticus	2.182
(++)(A)	AG2 (0-0.1 m)	Acinetobacter calcoaceticus	<u>2.104</u>	Acinetobacter calcoaceticus	2.036
(++)(A)	AG1 (0.2-0.3 m)	Acinetobacter calcoaceticus	<u>2.253</u>	Acinetobacter calcoaceticus	2.206
(+++)(A)	AG2 (0.2-0.3 m)	0.3 m) Acinetobacter calcoaceticus		Acinetobacter calcoaceticus	2.327
(++)(A)	AG3 (0.2-0.3 m)	3 (0.2–0.3 m) Acinetobacter calcoaceticus		Acinetobacter calcoaceticus	1.987
(+)(B)	FL1 (0-0.1 m)	L1 (0–0.1 m) Serratia proteamaculans		Serratia liquefaciens	1.885
(+)(B)	FL2 (0-0.1 m)	Stenotrophomonas rhizophila	<u>1.713</u>	not reliable identification	1.365

Notes: AL - arable land, PG - permanent grasslands, AG - abounded grasslands, FL - forest land.

T a b l e 2. MALDI-TOF results at Liptovská Teplička in autumn 2014.

Analyte Name	Analyte ID	Organism (best match)	Score Organism Value (second best match)		Score Value
(+++)(A)	AG1 (0-0.1 m)	Serratia fonticola	2.318	Serratia fonticola	2.086
(++)(B)	AG2 (0-0.1 m)	Serratia quinivorans	2.14	Serratia proteamaculans	2.013
(++)(A)	AG3 (0-0.1 m)	Serratia plymuthica	2.149	Serratia plymuthica	2.122
(+++)(A)	AG1 (0.2-0.3 m)	Serratia plymuthica	2.31	Serratia plymuthica	2.231
(++)(A)	AG2 (0.2-0.3 m)	Solibacillus silvestris	2.103	not reliable identification	1.468
(++)(A)	AG3 (0.2-0.3 m)	Myroides odoratus	2.297	Myroides odoratus	2.281
(+)(B)	FL1 (0-0.1 m)	Bacillus pumilus	1.905	Bacillus pumilus	1.89
(++)(B)	FL2 (0-0.1 m)	Viridibacillus neidei	2.247	Viridibacillus arvi	2.02
(++)(A)	FL3 (0-0.1 m)	Serratia quinivorans	2.168	Serratia proteamaculans	1.999
(+)(B)	FL4 (0-0.1 m)	Solibacillus silvestris	vestris <u>1.951</u> not reliable identification		1.513
(++)(A)	FL5 (0-0.1 m)	Serratia plymuthica	2.197 Serratia plymuthica		2.152
(+)(B)	FL6 (0-0.1 m)	Viridibacillus neidei	1.872 not reliable identification		1.67
(+)(B)	FL7 (0-0.1 m)	Serratia quinivorans	1.854	Serratia liquefaciens	1.747

Notes: AG - abounded grasslands, FL - forest land.

was *Acinetobacter calcoaceticus* that was probably determined by calcareous substrate (dolomitic limestone). The genus *Acinetobacter* is defined as including gram-negative coccobacilli that are strictly aerobic with a high ability to utilize phenol as the sole source of carbon and energy (Bergogne-Bérézin, Towner, 1996). Thus, phenolic compounds can be degraded by this microbe, and thus, remediate phenol contaminated soil. Most *Acinoteobacter* strains can grow in a simple mineral medium (Cordova-Rosa et al., 2009).

T a ble 3. MALDI-TOF results at Tajov in autumn 2015.

Analyte	Analyte	Organism	Score	Organism	Score
Name	ID	(best match)	Value	(second best match)	Value
(+)(B)	PG1 (0-0.1 m)	Bacillus cereus	1.846	Bacillus cereus	1.824
(+)(B)	FL1 (0-0.1 m)	Bacillus thuringiensis	1.976	Bacillus cereus	1.751
(+)(B)	FL1 (0.2-0.3 m)	.3 m) Bacillus weihenstephanensis		Bacillus thuringiensis	1.919
(+)(B)	B) FL2 (0-0.1 m) Bacillus thuringiensis		1.936	Bacillus cereus	1.903
(+)(B)	FL2 (0.2-0.3 m)	Bacillus cereus	1.97	Bacillus thuringiensis	1.947

Notes: PG – permanent grasslands, FL – forest land.

T a ble 4. MALDI-TOF results at Tajov in spring 2015.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(+)(B)	PG1 (0-0.1 m)	Pseudomonas chlororaphis	Pseudomonas chlororaphis <u>1.979</u>		1.964
(++)(B)	PG2 (0-0.1 m)	Pseudomonas libanensis <u>2.159</u> Pseudom		Pseudomonas fluorescens	2.081
(+)(B)	PG3 (0-0.1 m)	Pseudomonas brassicacearum	onas brassicacearum <u>1.847</u> Pseud		1.804
(++)(A)	PG4 (0-0.1 m)	Pseudomonas fluorescens	udomonas fluorescens <u>2.056</u> Pseu		1.87
(+)(B)	PG5 (0-0.1 m)	(0–0.1 m) Pseudomonas extremorientalis <u>1.874</u> I		Pseudomonas chlororaphis	1.837
(+)(B)	PG1 (0.2-0.3 m)	Pseudomonas chlororaphis	1.815	Pseudomonas koreensis	1.804

Notes: PG – permanent grasslands, FL – forest land.

T a b l e  $\,$  5. MALDI-TOF results at Tajov in autumn 2014.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(+)(B)	PG1 (0-0.1 m)	Bacillus cereus	1.846	Bacillus cereus	1.824
(+)(B)	FL1 (0-0.1 m)	Bacillus thuringiensis	1.976	Bacillus cereus	1.751
(+)(B)	FL2 (0-0.1 m)	Bacillus weihenstephanensis	1.951	Bacillus thuringiensis	1.919
(+)(B)	FL3 (0-0.1 m)	Bacillus thuringiensis	1.936	Bacillus cereus	1.903
(+)(B)	FL4 (0-0.1 m)	Bacillus cereus	1.97	Bacillus thuringiensis	1.947

Notes: PG – permanent grasslands, FL – forest land.

# **Explanatory Notes:**

Range	Description	Symbols
2.300 3.000	Highly probable species identification	(+++)
2.000 2.299	Secure genus identification, probable species identification	(++)
1.700 1.999	Probable genus identification	(+)
0.000 1.699	Not reliable identification	(-)

# Meaning of Consistency Categories (A - C):

Category	Description
A	<b>Species Consistency:</b> The best match was classified as "green" (see above). Further "green" matches are of the same species as the first one. Further "yellow" matches are at least of the same genus as the first one.
В	<b>Genus Consistency:</b> The best match was classified as "green" or "yellow" (see above). Further "green" or "yellow" matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
С	<b>No Consistency:</b> Neither species nor genus consistency. (Please check for synonyms of names or microbial mixture.)

Stenotrophomonas species have an important ecological role in the element cycle in nature (Ikemoto et al., 1980). Growth takes place at 4–37 °C. They are resistant to many antibiotics, for example, penicillin, tobramycin, imipenem and ceftazidime but susceptible to chloramphenicol, kanamycin and trimethoprim, sulfamethoxazole. Strains were plant-associated and isolated from the rhizosphere of oilseed rape and from the rhizosphere and geocaulsophere (tuber) of potato. Endophytic colonization was found (Wolf et al., 2002). Colonies of Stenotrophomonas rhizophila are yellowish. The strain uses xylose as a carob source. Antagonistic activity was shown against plant-pathogenic fungi. They are not active against bacteria (Wolf et al., 2002).

A novel estuarine bacterial strain, *Solibacillus silvestris* AM1, produces an extracellular, thermostable and fibrous, glycoprotein bioemulsifier (BE-AM1). Cell-bound BE-AM1 production by *S. silvestris* AM1 during the mid-logarithmic phase of growth coincided with a decrease in cell surface hydrophobicity, and an increase in cell autoaggregation and biofilm formation. Markande and Nerurkar (2016) study has revealed that the BE-AM1, a bacterial bioemulsifier, is a functional amyloid and has a role in biofilm formation and cell surface modulation in *S. silvestris* AM1.

Pseudomonas species are important decomposers of organic matter in soil, but are also pathogens in plants, animals and humans (Palleroni, 1993). The genus *Pseudomonas* was previously a heterogenous group of species, defined by a limited number of phenotypic characters. At present, the genus based on phylogenetic rRNA homology studies, several groups formerly belonging to Pseudomonas have been reclassified (Kersters et al., 1996). During the past decade, several new Pseudomonas species have been described. Pseudomonas fluorescens is an obligate aerobe, with an extremely versatile metabolism, and can be found in the soil and in water, is non-pathogenic (Frank, 1997). In common, the most of in spring-soils samples obtained strains were endospore forming, psychrotolerant species, like *Pseudomonas*, which correspond with climate, seasonal and geographical conditions, low temperature in the environment. P. frederiksbergensis pertaining to Frederiksberg near Copenhagen, Denmark, from where the organism was isolated. Cells are gram-negative, motile, non-sporeforming rods. Colonies are smooth and pale yellowish on agar. The species is oxidase- and catalase-positive, denitrifying, and shows hydrolysis of gelatin and accumulation of PHB. The species shows no acidification of glucose or hydrolysis of starch (Andersen et al., 2000). Serratia proteamaculans improve plant growth and/or nitrogen fixation in legume plants.

# Soil chemical properties

The differences in microbial diversity between both study sites and different land uses are conditioned besides land management also by natural factors including soil chemical parameters. Clay content is higher at TA (33.78% in AL) than at LT (26.72% in AL). The status of the AWCD and selected soil chemical parameters are listed in Tables 6 and 7 for LT and TA, respectively.

Rendzic Leptosol at LT is developed on dolomitic limestones that reflects in neutral pH values ranging from 6.86 in PG (depth 0–0.1 m) to 7.02 in FL (depth 0–0.1 m). The amount of organic carbon and nutrient varied. The higher content of organic carbon and nutrients

T a b l e 6. AWCD data and soil chemical parameters (average ± standard deviation) at Liptovská Teplička.

Depth	Land	AWCD	pH KCl	TOC	N <sub>t</sub>	P	К	Mg (mg·kg <sup>-1</sup> )
(m)	use			(g⋅kg <sup>-1</sup> )	(g⋅kg <sup>-1</sup> )	(mg·kg <sup>-1</sup> )	(mg·kg <sup>-1</sup> )	
	AL	0.685	$6.76 \pm 0.14$	$27.70 \pm 8.60$	$6.19 \pm 0.64$	$30.25 \pm 3.71$	110.66 ± 15.71	931.63 ± 62.18
0-0.1	PG	0.290	$6.86 \pm 0.22$	55.40 ± 8.59	$9.33 \pm 0.83$	$3.58 \pm 0.54$	99.61 ± 7.06	$1322.92 \pm 99.05$
0-0.1	AG	0.820	$6.99 \pm 0.35$	47.00 ± 7.94	$6.85 \pm 0.79$	54.53 ± 11.33	124.85 ± 14.73	$1049.49 \pm 110.06$
	FL	0.698	$7.02 \pm 0.32$	63.80 ± 4.49	9.63 ± 1.69	$9.35 \pm 3.70$	99.61 ± 12.01	1325.22 ± 140.50
	AL	0.433	$6.99 \pm 0.16$	21.20 ± 6.70	$3.81 \pm 0.62$	$25.32 \pm 4.24$	80.21 ± 6.70	$737.85 \pm 94.54$
0.2-0.3	PG	0.415	$6.87 \pm 0.21$	39.20 ± 7.31	$7.08 \pm 0.67$	$2.66 \pm 0.40$	80.21 ± 8.14	1144.25 ± 157.20
	AG	0.877	$6.97 \pm 0.21$	$23.20 \pm 4.93$	3.99 ± 1.27	45.93 ± 3.81	67.81 ± 9.78	708.81 ± 56.28

Notes: AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

T a ble 7. AWCD data and soil chemical parameters (average ± standard deviation) at Tajov.

Depth	Land	AWCD	pH KCl	TOC (g·kg <sup>-1</sup> )	N,	P (mg·kg <sup>-1</sup> )	K (mg⋅kg <sup>-1</sup> )	Mg (mg·kg <sup>-1</sup> )
(m)	use				(g⋅kg <sup>-1</sup> )			
0-0.1	PG	0.286	$4.98 \pm 0.40$	$41.10 \pm 7.93$	$7.59 \pm 0.57$	$1.85 \pm 0.19$	$104.72 \pm 13.77$	755.66 ± 429.04
0-0.1	FL	0.801	$5.06 \pm 0.46$	145.44 ± 11.33	$7.38 \pm 0.49$	$13.49 \pm 2.43$	$329.74 \pm 36.23$	717.57 ± 130.78
0.2.0.2	PG	0.597	$4.85\pm0.42$	$20.65 \pm 6.83$	$4.39 \pm 0.62$	$0.86 \pm 0.23$	$102.24 \pm 19.01$	782.73 ± 521.01
0.2-0.3	FL	0.661	$3.72 \pm 0.11$	32.42 ± 6.57	$2.16 \pm 0.47$	$1.25 \pm 0.38$	139.54 ± 16.38	$700.32 \pm 74.69$

Notes: PG - permanent grasslands, FL - forest land.

are in the depth of 0-0.1 m compared to the depth of 0.2-0.3 m. The highest total organic carbon content in the depth of 0-0.1 m is in FL ( $63.80~g\cdot kg^{-1}$ ) with the second highest AWCD values. The highest AWCD value was recorded in AG with lower TOC content but higher available phosphorus and potassium content. Because of the dolomitic substrate, the soil is rich in magnesium. Overall, the content of P and K are relatively low according to the evaluation of nutrient content in soil realized by the Central Control and Testing Institute in Agriculture in frame of agrochemical testing of soil (CCTIA, 2013).

Soil reaction in Haplic Cambisol, dystric variety, at TA ranged from 3.72 in FL (depth 0.2-0.3 m) that means extremely acid soil reaction to 5.06 in FL (depth 0-0.1 m) what is strong acid soil reaction. The higher content of organic carbon and nutrients are in the depth of 0-0.1 m compared to the depth of 0.2-0.3 m. The amount of organic carbon is substantially higher in FL compared to PG. Similarly, the AWCD values are higher in FL compared to PG. The soil in FL and PG is rich in magnesium, and poor in phosphorous. Potassium content is low in PG according to the evaluation of nutrient content in soil realized by the Central Control and Testing Institute in Agriculture in frame of agrochemical testing of soil (CCTIA, 2013).

## Soil biotic properties

The differences in microbial diversity can be affected by other living organisms in soil. We observed the numbers and fresh body biomass of earthworms and soil arthropods at LT and TA in spring 2015 (Table 8) (Kanianska et al., 2016) to observe the possible interactions with soil microorganisms.

T a b l e 8. AWCD data and averaged soil biotic parameters at Liptovská Teplička and Tajov.

Study site	Land use	AWCD	Earthworm biomass (g·m <sup>-2</sup> )	Earthworm density (ind.m <sup>-2</sup> )	Arthropod biomass (g·trap <sup>-1</sup> )	Arthropod density (ind.trap <sup>-1</sup> )
	AL	0.685	7.23	20.99	13.84	32.71
LT	PG	0.290	28.20	74.60	3.08	23.29
	AG	0.820	5.10	9.00	3.23	35.28
	FL	0.698	0.70	3.30	0.82	6.42
	PG	0.433	40.80	108.50	1.53	31.43
TA	FL	0.415	9.40	18.70	2.85	21.86

Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

At both study sites, the highest earthworm density and body biomass within different land uses were observed in PG. In LT, the highest arthropod biomass and density were observed in AL. In TA, the higher arthropod biomass was in FL but the arthropod density was higher in PG.

### Discussion

Microbial activity and land management

Land management reflected in the microbial activity. In AL at LT, we observed relatively high microbial activity. The area is under organic farming that assumes sustainable land management.

Agricultural practices have proven to be unsuitable in many cases, causing considerable reductions in soil quality. Land management practices can provide solutions to this problem and contribute to get a sustainable agriculture model. García-Orenes et al. (2013) studied the effect of different agricultural management practices on soil microbial community structure. Their results showed a substantial level of differentiation in the microbial community structure, in terms of management practices, which was highly associated with soil organic matter content. The microbial community composition of the abandoned agricultural soil was characterised by increases in both fungal abundances and the metabolic quotient, suggesting an increase in the stability of organic carbon. The ratio of bacteria:fungi was higher in wild forest coverage and land abandoned systems, as well as in the soil treated with oat straw. Similarly, in our study, the abandoned grasslands and forest land were typical in higher microbial activity than the managed permanent grasslands mown for hay.

## Microbial activity and soil chemical properties

Microbial activity is affected by various abiotic factors. Prokaryotic communities in soils are among the most taxon rich of any microbial habitat (Madigan, 2008), and the abiotic heterogeneity in soils is a major contributor to their biological diversity (Fierer, Lennon, 2011).

Salinity and pH are the major drivers of microbial communities in all habitats and, especially, in soils (Herlemann, 2011). It is expected that agricultural land use change may impact soil microbial community composition and biomass, primarily by soil properties such as pH, soil depth, moisture and temperature (Lauber et al., 2009). In our research, at LT study site, the highest microbial activities were observed in AG followed by FL with higher pH values than in the other two land-uses (Table 6). At the TA study site, the higher pH value was observed in FL with higher microbial activity than in PG (Table 7).

The distribution of bacteria also depends on the content of organic carbon (OC) and clay (Ranjard et al., 2000). The surfaces of micro and macro aggregates differ in their physicochemical properties and provide habitats for soil microorganisms (Ditterich et al., 2016), frequently attached to mineral surfaces or organo-mineral complexes. Ditterich et al. (2016) showed that changes in substance availability as well as mineral properties are important drivers for the development of microbial communities. The ability of a few soil bacteria to transform unavailable forms of phosphorus (P) and potassium (K) to an available form is an important feature in plant growth (Jat et al., 2015; Kumar et al., 2016). In our research, higher AWCD values indicate relations between higher microbial diversity and higher nutrient availability.

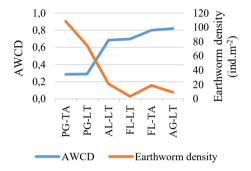
Soil (represented by AL) at the TA study site has higher content of clay as compared to the soil at LT. FL at the TA study site also has the highest total organic carbon content within all land-uses (Table 6). High AWCD data (0.801) indicate high microbial activity that can be determined by these two factors. On the other hand, in the samples of Klin and Mutne peatlands /Slovakia/ high share of genera *Bacillus flexus*, *Serratia liquefaciens*, *Pseudomonas proteolytica*, *P. fragi*, *P. chlororaphis* were identified (Júdová et al., 2015), similar to the permanent grasslands and forest lands at Tajov (Tables 3, 4). Klin peatland is bog-forest type and Mutne is active raised bogs with a mosaic of alkaline fens, threatened by succession and decreasing underground water level.

Besides the abiotic factors, plants exert strong controls on the composition of bacterial communities in vegetated soils (Zak et al., 2003). Also, our results showing differences in AWCD data (Tables 6, 7) can be determined not only by chemical and physical soil parameters but also by plant composition as a consequence of different land use types. There is also a possibility that within one land use type, the effect of different plants is recorded. Urbanová et al. (2015) have reported that the effects of the tree species in a forest ecosystem explain a large proportion of variation in microbial community composition than other soil properties, especially in fungi.

# Microbial activity and soil biotic parameters

Microbial activity is affected by the activity of other groups of organisms living in soil. The biochemical decomposition of OM (organic matter) is primarily accomplished by microorganisms, but earthworms are crucial drivers of the process, as they may affect microbial decomposer activity by grazing directly on microorganisms (Aira et al., 2009), and by increasing the surface area available for microbial attack after comminution of OM (Domínguez et al., 2010). Some microorganisms may be a source of food for earthworms, but the amounts

consumed and the ability of earthworms to digest and assimilate microbial biomass vary with earthworm species, its ecological category, food substrate and the environmental conditions in which the earthworms are living (Brown, Doube, 2004). In our study, we found out such possible effects of earthworms on microorganisms. Figures 3 and 4 show mutual interactions between soil activity expressed by AWCD values and earthworm density and biomass. The higher microbial activity is connected with the lower earthworm density and biomass in the depth 0–0.1 m.



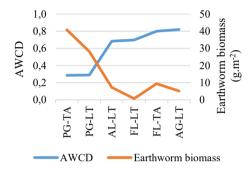


Fig. 3. Average well-colour development (AWCD) and earthworm density at Liptovská Teplička and Tajov. Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

Fig. 4. Average well-colour development (AWCD) and earthworm biomass at Liptovská Teplička and Tajov. Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

### Conclusion

At both study sites, we found the differences in the microbial activity conditioned by the different land use. There were found different strain of microorganisms like *Acinobacter calcoaceticus*, *Stenophomonas rhizophila*, *Solibacilus silvestris*, *Pseudomonas fluorescens*, *P. frederiksbergensis*, *Seratia proteamaculans*. The highest microbial activity deduced on AWCD values in the depth of 0–0.1 m was observed at the TA study site in FL with higher TOC, available P and K content. At the LT study site, the highest microbial activity was recorded in AG followed by FL and organically managed AL. Higher AWCD values indicate relations between higher microbial diversity and higher nutrient availability at both study sites. In the comparison of land use types, permanent grasslands showed the lowest microbial activity in the depth of 0–0.1 m. But in the depth of 0.2–0.3 m in PG of both study sites, the higher microbial activity was recorded as compared to the depth of 0–0.1 m. In addition, lower AWCD values in PG were in line with the lower available P and K content but higher earthworm density and biomass.

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