Functional diversity of soil microorganisms in the conditions of an ecological farming system

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Abstract

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In the current study, we investigate the relationships among the soil functional diversity, physicochemical properties and heavy metals presence in an ecological farming system. The soil samples were collected from permanent research sites, from A soil horizons, at a depth from 0.05 m to 0.15 m, in June 2018. In fresh soil samples, we evaluated the metabolic profiles of their microbial communities, using Biolog® Eco Plates. The research showed that the soil physical properties got adjusted after a long-term application of an ecological farming system and that the measured values were stabilised, reaching the levels comparable with the average values for the relevant soil type. It is necessary to devote a continual attention to soil reaction, because soil is naturally acidified through acid atmospheric fallout as well as through calcium uptake-off by plants. The values of the selected heavy metals in the monitored period did not exceed the limit values specified in the Act No. 220/2004 Coll. Based on the results of Shannon's diversity, we can conclude that the diversity in the investigated sites was low, from moderate to medium. The differences (3.26–3.36) among all 11 study localities were very small and not significant. There were determined the correlations between the soil functional diversity, soil physicochemical properties, and heavy metal contents. The average well colour development (AWCD) positively correlated with soil reaction and with Mg content and significantly negatively correlated with contents of Hg, Zn and Cu; equitability significantly positively correlated with soil reaction, Mg, AWCD and Shannon's diversity. Spearman's correlation coefficients confirmed the positive correlation between Shannon's diversity and soil reaction, AWCD and Mg. In our study, no correlation was found between the functional diversity of microorganisms and the soil physical properties.

Keywords

AWCD*,* Biolog® system, bulk density, heavy metals, porosity, soil reaction

Introduction

Soil environment is a very complex and considerably diverse biological community providing a wide range of services for soil ecosystems. This is strategic for sustainable life of natural and managed ecosystems. The most active component of the soil biocenosis is microorganisms whose role in soil ecosystems is of key importance. Soil biodiversity is probably the essential factor for maintaining ecosystem functions in a disturbed environment. Soil biodiversity can be measured directly (species richness) or indirectly, through standardized procedures (various indexes). The state of the soil environment is, in addition to the soil activity, associated with the soil biota diversity. In terms of biology, soils belong to the most diverse habitats on the Earth. It has been estimated that 1 g of soil contains up to 1 billion bacteria cells comprising tens of ten-thousands of taxa, up to 200 m fungal hyphae,

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and a wide range of nematodes, earthworms, and arthropods (Bardgett and Van der Putten, 2014). Numerous studies have shown that intensive land use threatens soil biodiversity; simultaneously, land-use intensification and associated reductions in soil biodiversity contribute to several environmental problems, such as the eutrophication of surface water, reduced aboveground biodiversity, and global warming. This all can negatively affect human well-being (Bender et al., 2016).

A series of physical, chemical and biological indicator parameters are used to assess soil health, and quality and changes in ecosystem sustainability (Avellaneda-Torres et al., 2018). Monitoring the structure of microbial communities and animals is a sensitive tool for assessment of soil quality and health. Generally, the microbial parameters in the soil ecosystems are considered to be the best indicators reflecting changing soil quality and properties, and thus, enabling early detection of soil degradation (Romaniuk et al., 2011; Macci et al., 2012). Spatial microbiological parameters, in particular, microbial biomass, basal respiration and the structure of microbial communities (Doran and Zeiss, 2000), were suggested as possible indicators of soil quality and were used in national and international monitoring (Yao et al., 2000). The assay is based on the Biolog system using 31 different carbon sources to produce a metabolic profile of microorganisms (Garland and Mills, 1991).

Long-term sustainability concerns are growing in agriculture owing to over- and under- application of fertilizers and owing to poor management of available resources. This results in soil health deterioration and declining crop productivity. Balanced and integrated use of organic and inorganic fertilizers is the most logical concept for managing and sustaining long term soil health and crop productivity (Meena et al., 2019). Wamsley and Skleničkа (2017) reported that insufficient motivation of farmers to invest in soil conservation practices can initiate soil degradation, followed by an overall lower biological activity. Higher environmental quality, ecological farming system and the concept of ecological intensification have become essential parts of the sustainable development strategies related to soils in many countries (Liao et al., 2007). It appears that increased soil biodiversity increases the sustainability and stability of ecosystems (Bender et al., 2016). Choudhary et al. (2018) notes in its study that monoculture cultivation leads to a decrease of soil quality and biota. Sustainable intensification of cultivation systems is the best alternative to improve the soil quality index and to protect natural resources.

At the present time, there is little knowledge about soil development in the conditions of ecological farming systems, running on principles of soil maintenance aimed to increase the soil natural productivity, following the natural cycle as close as possible, with the highest possible reduction of external, mainly energetic, and chemical inputs (Duguma et al., 2010; Fazekašová, 2012). The present findings are difficult to compare due to the different soil-ecological conditions in which they were obtained.

The objectives of this study were (i) to analyse selected physical and chemical properties and heavy metal contents in soil; (ii) to assess the level of biodiversity of soil ecosystems, using the metabolic potential analysis by the Biolog system; (iii) to study the relationship between soil functional diversity and soil physicochemical properties and heavy metals in the conditions of an ecological system of soil farming.

Materials and methods

Study area

The investigated area Liptovská Teplička (48°57'39.3''N, 20°05'49.9"E) is situated in the marginal region of north-eastern Slovakia. The ecological farming system has been applied here since 1996. The area of Liptovská Teplička is situated in the Low Tatras National Park at an altitude ranging from 846 to 1,492 m asl. In terms of geomorphological division, it is a part of the sub-assemblies of the Kráľovohoľské Mts (Mazúr and Lukniš, 1980). The whole area is situated in the mild zone with the sum of average daily temperatures above 10 ºC ranging from 1,600 to 2,000 and the average precipitation of 700–1200 mm (Klimatický atlas Slovenska. Climate atlas Slovakia, 2015). The soil conditions are relatively homogeneous, the largest area being represented by Cambisols, mostly moderate and strongly skeletal, mainly in the subsoil. The second most common type of soil is Rendzic, moderate, shallow, and skeletal. In addition, Histosols occur in this territory. From the point of view of relief, the majority of the land is situated on the slopes. In the current crop structure, cereal acreage represents 33.3%, potatoes 16% to 18% and fodder crops 49.8%. Arable land is fertilised with a manure dosage of approximately 30 t ha^{-1} once in two years.

Soil sampling, analyses and statistical analyses

Soil samples were collected on 11 permanent research sites of Liptovská Teplička with ecological farming system, from A horizons, a depth of 0.05 m to 0.15 m (Fig. 1) in June 2018.

After homogenization, soil samples were manually crumbled, dried at room temperature, sieved (≤2 mm) and stored in polyethylene bags until their analysis. We evaluated soil reaction in $CaCl_2$ solution (5 g of soil mixed with 25 ml of 0.01 mol $CaCl_2$) using a pH meter Mettler Toledo. The total contents of Cd and Hg were determined by atomic absorption spectrometry (AAS) and the total contents of Cu, Pb, Zn, Cr, Mn and Mg were determined by X-Ray fluorescence spectrometry following the methodology by Fiala et al. (1999). The assessed values of heavy metals in soils were compared to the limit values of Slovak soils (Act No. 220/2004). From physical soil parameters, we studied and evaluated the bulk density (t m^{-3}) and porosity (%) in Kopecky physical cylinder with

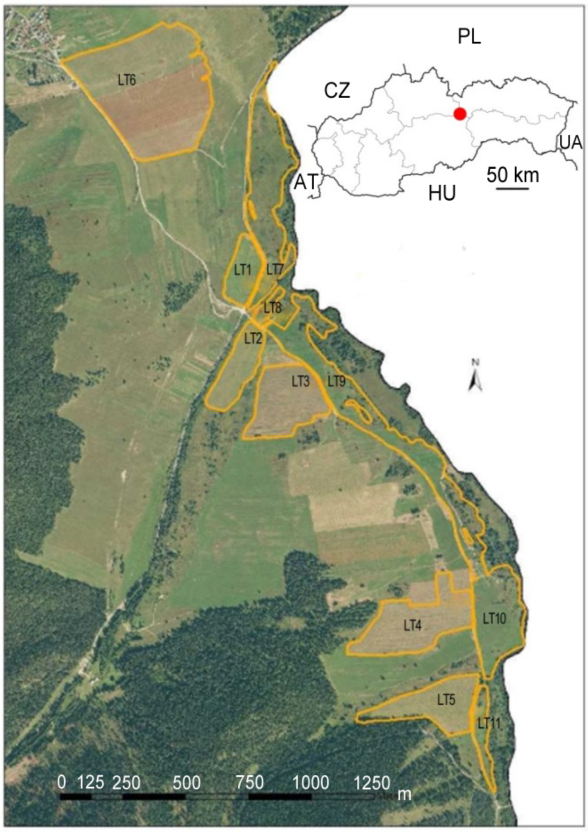

Fig. 1. The localization of sampling sites in Liptovská Teplička (Slovakia).

a capacity of 100 cm[3] (Fiala et al., 1999). In fresh soil samples, we evaluated metabolic profiles of microbial communities using Biolog® Eco Plates (Garland, 1997; Hofman et al., 2004). Microtiter plates with 31 different organic substrates were incubated with 150 µl of the extract from the sample (dilution 1 : 10,000 in 0.9% NaCl) at 27 °C for 10 days. During the incubation of the samples, we used a device ELx808 TM Absorbance Microplate Reader to determine daily the value of the absorbance at 590 nm corresponding to the activity of microorganisms on various substrates. The data normalized parameter AWCD (average well colour development) was calculated according to Garland (1997) and functional diversity of soil microbiological communities was calculated for BIOLOG data by classical Shannon diversity index (H‘)

$$H' = -\sum_{i=1}^{s} \frac{xi}{N} log2 \frac{xi}{N}$$

(Shannon, 1948). The results were evaluated based on scales: 1 extremely low (<0.5), 2 very low (0.5–1.0), 3 medium low (1.0–1.7), 4 low (1.7–2.5), 5 low to moderate (2.5–3.3), 6 medium (3.3–4.0), 7 moderately high (4.0–5.0), 8 high (5.0–7.0), 9 very high (7.0–10.0) and 10 extremely high (>10.0).

Schematic diagram of the research methodology is shown in Fig. 2. The obtained data were processed statistically by the means of the STATISTICA 13 software and PAST 3. The level of significance between the soil properties was calculated using Spearman's correlation coefficient. The data were LOG transformed, prior to the analysis.

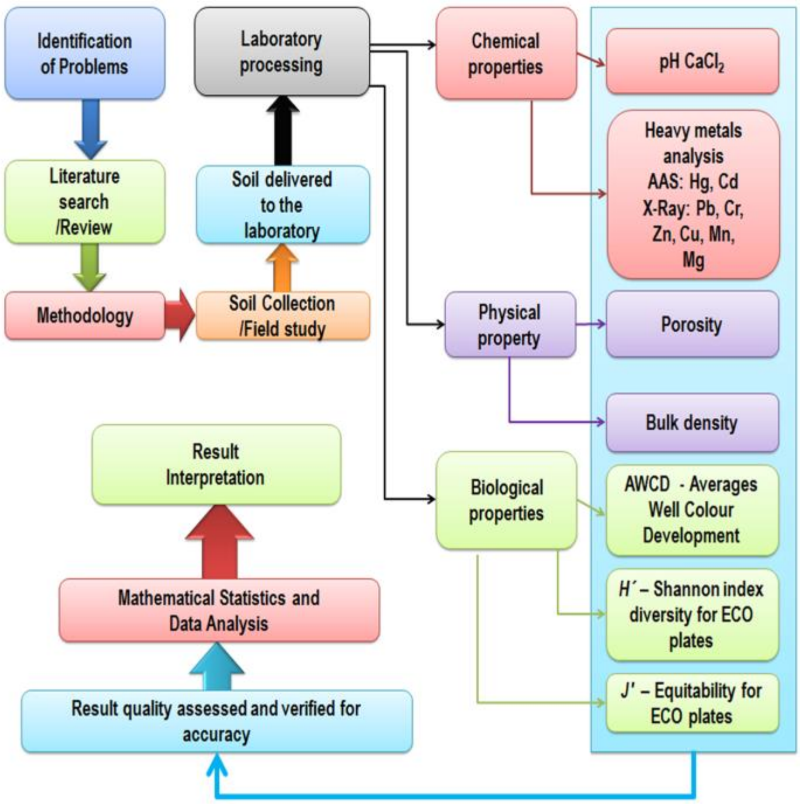

Fig. 2. Schematic diagram of the research methodology.

Results and discussion

The descriptive statistics of the soil parameters are listed in Table 1. Soil reaction is considered to be one of the main chemical properties, as it affects all biochemical reactions in the soil environment (Hohl and Varma, 2010). Soil pH is a primary factor controlling the bacterial diversity and its community composition (Bargdett, 2005; Shen et al., 2013; Yang et al., 2019). The soil reaction in the investigated area with an ecological farming system ranged between 5.40 ± 0.76 (median ± standard deviation), the range of values of the exchange soil reaction defines the soil as extremely acidic to neutral.

A commonly used soil physical characteristic expressing soil structural quality is soil bulk density (ρ_d). This parameter is often used in agronomic studies, as it indicates the presence of compacted layers. As such, it is commonly considered as a suitable trait for efficient measurement of soil carbon and nutrient stocks (Bondi et al., 2018). There is only little knowledge about bulk density, since the measurement of this parameter is demanding, as it is pointed out in the work by Premrov et al. (2017).

Bulk density as an integral value of soil granularity, humus content and anthropogenic impacts on soil should not exceed the limits given for the individual soil types. Soil total porosity is the best indicator of soil structure quality, as the porosity can also influence soil microbial communities (Pagliai and Vignozzi, 2002).

Total porosity is closely related to bulk density. The total pore volume values should not fall below 38% for sandy soil and below 48% for clay-loam soil (Líška et al., 2008). A long-term research has shown that ecological soil farming regulates bulk density of soil. The measured values of the bulk density were within the range 1.09 ± 0.11 (t m^{-3}). The values listed in Table 1 show that the porosity levels ranged within 57.32 ± 4.42 (%), indicating optimum conditions created for the growth of most arable crops (defined with an interval from 55 to 65%).

The evaluation showed that the content of heavy metals in soil with an ecological farming system did not reach the maximum values legalised for the Slovak Republic (Act No. 220/2004 Coll.) and that the measured values corresponded with the natural contents of the surveyed elements in soil and in base rocks. At the same time, in ecological systems, no anthropogenic pollution caused by applying chemical substances and sediments in soil is present (Čurlík and Šefčík, 1999; Makovníková et al., 2006).

For the analysis of changes in microbial communities, we used BIOLOG® Eco Plates. The most significant advantage of the Eco plates is that they contain 31 substrates (Garland, 1997). To calculate the Shannon diversity index (Shannon, 1948), we used the absorbance at a given AWCD (average well colour development) in the samples investigated after 168 hours. The AWCD is an important and sensitive indicator reflecting the metabolic profiles of the soil microbial community. After 24 h of incubation, the rate of substrate utilization (colour development) at each site was accelerated for all soils. Figure 3 shows the results for the investigated soil samples. During 168 hours, we found out differences in the ability of microorganisms to metabolize the substrates. The highest

Table 1. Values of selected soil parameters, heavy metals, and average well colour development (AWCD) in Biolog® Eco Plates in the investigated areas Liptovská Teplička (Slovakia)

Soil parameter	Mean	Min	Max	Median	Standard deviation
pH/$CaCl_2$	5.51	4.40	6.90	5.40	0.76
ρ_d (t m^{-3})	1.07	0.86	1.22	1.09	0.11
Po (%)	59.31	54.08	67.49	57.32	4.42
Hg (mg kg^{-1})	0.10	0.07	0.12	0.10	0.02
Cd (mg kg^{-1})	0.32	0.09	0.70	0.30	0.19
Pb (mg kg^{-1})	15.60	2.37	23.97	17.57	7.15
Cr (mg kg^{-1})	55.64	42.00	71.00	57.00	9.58
Zn (mg kg^{-1})	129.41	82.63	293.70	106.47	63.85
Cu (mg kg^{-1})	9.76	7.58	14.69	8.87	2.22
Mn (mg kg^{-1})	736.36	500.00	900.00	800.00	136.18
Mg (mg kg^{-1})	441.73	264.00	898.00	303.00	237.38
AWCD	1.67	1.32	1.84	1.76	0.16
H'	3.31	3.26	3.36	3.33	0.04
J'	0.97	0.95	0.98	0.97	0.01

ρ_d – bulk density, po – porosity, H´ – Shannon index diversity for ECO plates, J' – equitability for ECO plates, AWCD – average well colour development, limit value Act No. 220/2004 Coll.: Hg – 0.5 mg kg^{-1}, Cd – 0.7 mg kg^{-1}, Pb – 70 mg kg^{-1}, Cr – 70 mg kg^{-1}, Zn – 150 mg kg^{-1}, Cu – 60 mg kg^{-1}.

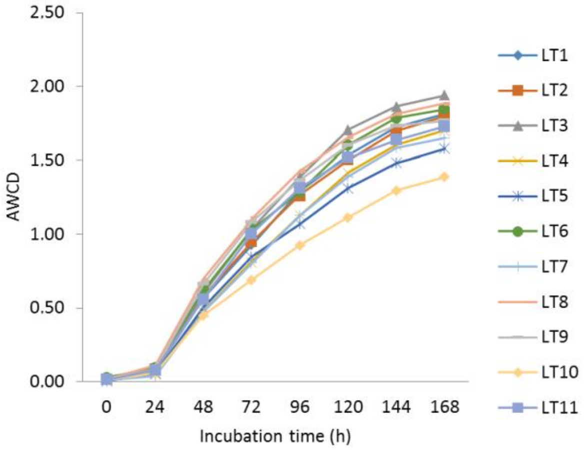

Fig. 3. Average well colour development (AWCD) of Biolog® Eco Plates (31 substrates) in the investigated area Liptovská Teplička (Slovakia).

Table 2. Spearman's correlation between functional diversity of microorganisms and physical soil parameters, chemical soil parameters and heavy metals

	AWCD	*H′*	*J′*
pH/$CaCl_2$	0.60**	0.51**	0.55**
ρd	0.18	0.01	–0.02
Po	–0.18	–0.02	0.02
Hg	–0.45*	–0.27	–0.31
Cd	–0.13	–0.04	–0.06
Pb	–0.08	0.00	–0.01
Cr	–0.17	–0.28	–0.25
Zn	–0.39*	–0.29	–0.30
Cu	–0.46*	–0.30	–0.32
Mn	–0.22	–0.16	–0.14
Mg	0.56**	0.45*	0.50**
AWCD		0.90**	0.90**
H′			0.99**

average metabolic activities of the community of microorganisms (AWCD) values were found out on the locality LT3 (Fig. 1). Histosols of this locality, consist primarily of organic materials. Based on the results of Shannon index, we can conclude that the diversity in the investigated site is low, from moderate (2.5–3.3) to medium (3.3–4.0). The differences (3.26–3.36) among all 11 localities were very small and not significant (Table 1). Similarly, Gömöryová et al. (2016) did not find any changes in microbial characteristics between the forest floor horizons. Higher diversity stabilizes the ecosystem's functional properties, improving them more stable, more productive, and resistant to stress factors and disturbances (Torsvík and Øvreas, 2002). Gałązka et al. (2017) state that management practises and seasons were two important factors affecting soil microbial communities. Therefore, further research will be needed to identify the key relationships between soil parameters.

Table 2 shows the correlation relationships between the functional diversity of microorganisms and physical soil parameters, chemical soil parameters and heavy metals. The AWCD was positively correlated with pH ($R = 0.60$, $p < 0.01$) and Mg ($R = 0.56$, $p < 0.01$) and significantly negatively correlated with Hg, Zn and Cu ($p < 0.05$). In addition, we found out significantly positive correlations between equitability-pH, Mg, AWCD and Shannon index diversity ($p < 0.01$). Spearman's correlation coefficients confirmed the positive correlation between Shannon index diversity-pH ($p < 0.01$), Shannon index diversity-AWCD ($p < 0.01$) and Shannon index diversity-Mg ($p < 0.05$). Many studies have noted, that soil pH was significantly and positively correlated with the soil bacterial diversity (Yang et al., 2019; Rousk et al., 2010).

Similar results were obtained by Zhu et al. (2017) who found out that Shannon-Wiener index positively correlated with AWCD. Yang et al. (2019) also found a negative correlation between pH and the soil bacterial diversity, but these correlations become negative at soil pH values higher than 8. In our research, the soil pH was 4.40 (min) to 6.9 (max), (Table 1). There are strong negative correlations between the heavy metal contents and the microbial parameters (Tischer et al., 2008). Vincent et al. (2018) note that the moderate contamination with heavy metals altered the taxonomic and functional composition of the soil biota. The same has been confirmed in our research, nevertheless, with the measured values corresponding with the natural contents of the observed elements occurring in soil and in base rocks. These results indicate that these variables are potentially good indices of soil environmental quality, which is in accordance with the study Gałązka et al. (2017).

Soil total porosity was also found out to be one of the main factors influencing the soil bacterial and fungal community compositions (Yang et al., 2019). This result is not consistent with our findings. In our study, no correlation has been identified between the functional diversity of microorganisms and the soil physical properties.

Conclusions

The research showed that the soil physical properties at the inspected locality got modified after a long-term application of an ecological farming system. The measured values were found stabilised, reaching the levels comparable with the average values for the relevant soil type. It is necessary to pay a continual attention to soil reaction, because soil is naturally acidified due to acid atmospheric fallout as well as due to calcium uptake-off by plants. The values of the selected heavy metals obtained in the monitored period did not exceed the limit values set in the Act No. 220/2004 Coll. Based on the results of Shannon index diversity, we can conclude that the diversity at the investigated site is low, from moderate to medium.

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