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Microplot long-term experiment reveals strong soil type influence on bacteria composition and its functional diversity

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ABSTRACT

Microbial taxonomic and functional diversity in eight different types of soils were determined by two independent techniques in the micro-plot experiment, where the collected soils were under the same agricultural management for over 100 years. This research aimed to discover the differences in bacteria composition affected directly by the soil type. The utilization level of 31 substrates measured by Biolog EcoPlate approach resulted in significant functional differences related to soil types and their physiochemical properties. Next Generation Sequencing method enabled to classify and correlate bacteria abundance to the soil functional properties, giving an opportunity to select genera characteristic to specific types of soil. By statistical methods, the soil core microbiomes were calculated, for five soils considered as good quality and three considered as acidic, poor quality. The research provided the basis for the search of soil marker bacteria and a better understanding of the relationships between soil physiochemical properties and the microorganisms inhabiting them. Moreover Shannon's diversity and evenness indexes calculated for both methods showed the same pattern and classified soils consistently. It proved that biochemical processes in the soil ecosystems are directly and undeniably related to microorganisms abundance and diversity.

1. Introduction

The soil ecosystem is primarily known for its supporting of crop production, which largely depends on the biochemical processes carried out by the micro-organisms inhabiting it (Girvan et al., 2003). The influence of factors such as vegetation period and agrotechnical treatments on the amount and activity of microorganisms, were investigated (Gałązka et al., 2017). Some studies show that the composition of soil microorganisms, including rhizosphere bacteria, depends to a large extent on the interaction with plants. On the other hand, some recent studies have shown that chemical, physical and geological properties of soil itself, such us: salinity, pH, type and its origin (Ma et al., 2010) have a greatest influence on the development of specific bacteria. For instance, it has been proven that a low pH can increase fungal abundance up to five times and decrease the number of bacteria in the soil (Rousk et al., 2009).

Two different and independent techniques were implemented to focus on microbial composition (Next Generation Sequencing, NGS) as well as on functional properties of each soil (Biolog EcoPlate approach). The EcoPlate method (Insam, 1997) has been used in many areas, including ecotoxicological studies (Gryta et al., 2014), or microbial metabolism shifts after inoculation with bacterial strains (Siczek and Lipiec, 2016). It was also a useful tool for tracking functional diversity changes correlated to antibiotic-associated disturbances in soil ecosystems (Liu et al., 2012). On the other hand, molecular methods such as sequencing make it possible to determine the composition of the microenvironment. However, this knowledge largely depends on the prior knowledge and completeness of the genomic reference databases that are still growing.

The isolation of the examined soils in the form of micro-plots allowed for a thorough examination of their properties, which were influenced only by the type of soils and their history. The purpose of this work was to investigate the effect of the soil type on the diversity of bacteria and its functional consequences.

2. Materials and methods

2.1. Field experiment

The research was conducted in 14 m^2 micro-plot experiment established in 1883 at the Institute of Soil Science and Plant Cultivation – State Research Institute in Pulawy, Poland (GPS coordinates: 51.415218, 21.960489). The experimental blocks consisted of eight soils of different origin (Table 1). Four soils are characterized by a high

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Table 1
Soil types used in the experiment.

Abbreviation	Soil type	pH (in H_2O)	Shannon's diversity (H) index (NGS)	Shannon's diversity (H) index (Biolog)	Eveness (E) (NGS)	Eveness (E) (Biolog)
MZ 5	Gleyic Chernozem	7.4	4.939	3.210	0.774	0.973
MZ 3	Fluvic Cambisol	7.5	4.924	3.200	0.766	0.960
MZ 2	Cambic Leptosol	7.3	4.983	3.260	0.798	0.979
MZ 7	Cambisol	5.6	4.910	3.160	0.776	0.958
MZ 4	Haplic Luvisol	5.4	4.807	3.010	0.759	0.959
MZ 1	Haplic Cambisol	4.7	4.822	2.820	0.749	0.911
MZ 8	Brunic Arenosol II	4.5	4.603	2.890	0.703	0.949
MZ 6	Brunic Arenosol I	4.0	4.462	2.430	0.695	0.877
Control	Haplic Luvisol	5.9	5.089	3.021	0.803	0.961

Abbreviations are based on experimental plots order.
Soil types are listed from the top quality to the worst.

organic carbon, pH 7.3–7.5 and good quality for plants cultivation: Cambic Leptosol, Fluvic Cambisol, Gleyic Chernozem and Cambisol. Other three soils are acidic (pH 4.0–4.7), characterized by a low organic carbon and poor quality for cultivation: Haplic Cambisol and two Brunic Arenosols (I, II). Finally, one soil, Haplic Luvisol, characterized by a low organic carbon and pH 5.4. Soil types classification and stability of selected physicochemical parameters over twenty years of microplots maintenance, are presented in Siebielec et al. (2015) work.

Each of the eight study soils were under the same weather conditions and agricultural management (hand tillage). Also the same plants were cultivated at the same time in each of microplots (barley, *Hordeum vulgare* L.) as recent crop. As an experiment control, the Haplic Luvisol soil from the Agricultural Experimental Station (AES) of Institute of Soil Science and Plant Cultivation in Grabow (Poland) was chosen. On the one hand, Haplic Luvisols are the most commonly found in Poland. The second advantage was the possibility to compare bacteria composition between an arable land under continuous cultivation and a semi-isolated soil from an experimental microplot. This stage allowed to confirm that despite long-term isolation of the soils in the form of microplots, the microbiological composition remained similar between the same types of soils. However, suitable soils could not be found as controls for other types of soil. For the other soil types, there were no equivalent controls at this stage of the study. Samples were collected in 2016, April (soil before sowing) from 15 representative spots per each soil type, at 0–20 cm depth. Next, soil samples were pooled, sieved through 2 mm sieve, and stored at −20 °C for future analysis.

2.2. Dehydrogenases activity and pH measurement

From each soil sample, 10 g were suspended in 20 ml sterile water and shaken for 30 min. Sedimentation was then carried out for 30 min. The pH was measured at room temperature. Dehydrogenases (DHs) activity measurement was performed by a 2,3,4-triphenyltetrazolium chloride (TTC) reducing, as described by Casida et al. (1964), with slight modifications. The soils were prepared in triplicates (6 g of each) and mixed with 60 mg of CaCO3. Then, 1 cm^3 of 3% TTC (w/v) and 2.5 cm^3 of distilled water were added. The samples were incubated in 37 °C for 24 h and subjected to extraction with ethyl alcohol (25 cm^3). The extracts were filtered through cellulose filter paper, and absorption was measured at 485 nm. The DHs activity was expressed as micrograms of triphenyl formazan (TF) per 1 g of soil dry weight after 24 h of incubation.

2.3. DNA extraction and next generation sequencing (Miseq, illumina)

Total DNA was extracted from the same amount (350 mg) of each soil sample with FastDNA™ SPIN Kit for Soil (MP Biomedical) according to manufacturer's instruction. Purity and concentration were measured in NanoDrop 1000 Spectrophotometer (Thermo Scientific). DNA was diluted with sterile MiliQ water to 10 ng/µl concentration and 20 µl was sent to Genomed S.A (Warsaw, Poland) for performing Next Generation Sequencing, targeting the hypervariable V3-V4 region of 16S rRNA gene, using the 341F and 785R primers (Klindworth et al., 2013). The library preparation was made with Q5 Hotstart HF DNA Polymerase (NebNext) according to the manufacturer's instruction. Sequencing type was paired-end on MiseQ apparatus and BaseSpace server (Illumina) with 16S Metagenomics (v1.0.1) protocol. Sequences shorter than 1250 bp and containing more than 50 degenerated bases were filtered out. Subsequently, the reads were demultiplexed and classified to taxonomic categories based on Greengenes 13.5 database, modified by Illumina. As a result, the taxonomic profiles of each soil sample were generated, including the number of reads per each taxa. In this paper, the genus level was chosen for calculations.

2.4. Biolog EcoPlate™ (Biolog inc., Hayward, CA, USA)

One gram of each soil sample was suspended in 99 ml of sterile water and vortexed for 20 min at room temperature. Then suspension was left to settle for 30 min at 4 °C (Weber and Legge, 2009). Each well of EcoPlate was inoculated by 120 ul of suspension and incubated at 28 °C for 144 h. Absorbance at 590 nm was measured every 24 h with a plate reader Biolog MicroStation™.

2.5. Statistical analysis

Using Biolog EcoPlate results and Next Generation Sequencing genera abundance, Shannon's diversity (H) (Hill et al., 2003) and evenness (E) (Magurran, 1988) indexes were calculated. The most abundant genera representatives and Biolog EcoPlate substrates optical densities were subjected to two-group correlation analysis in Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA, 2011) with $P < 0.05$ significant level. Average well color development (AWCD) was measured as a mean of optical densities from 31 wells (Garland, 1997). Each well absorbance was corrected by the subtraction of optical density of well containing the water.

The core microorganisms (Core Biome) were calculated in MEGAN V6.7.11 software (Huson et al., 2016) separately by dividing soils from this experiment into two groups. Group A included good quality soils: Gleyic Chernozem, Fluvic Cambisol, Cambic Leptosol, Cambisol and Haplic Luvisol. Group B consists of acidic, poor quality soils: Haplic Cambisol, Brunic Arenosol (I and II). Core Biome calculation parameters were as follows: sample threshold = 60% for Group A and 66% for Group B, which means that each core genus should exist in at least three of five good quality soils, and two of three poor quality soils; class threshold = 1%, which signifies that every genus which abundance was below 1% in a soil was filtered out. Principal Coordinates Analysis (PCoA) was calculated in Megan V6.7.11 software, taking into account also the control soil. The comparison was made on normalized, relative number of reads and presented as a percent of a total reads per sample. The genus level was chosed as the most accurate, based on the PCoA