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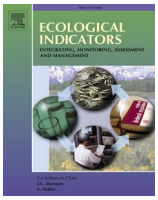

Original Articles

Microbial diversity as an indicator of a diversified cropping system for luvisoils in a moderate climate. Case study – Long term experiments from Poland

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ABSTRACT

The idea of agricultural sustainable in the EU is based on, both minimizing interference with the soil system as well as diversifying crop rotation what relates to the limited cultivation system (changed from plow to no-plowing tillage) as well as organic fertilization is often abandoned. Taking above into account, our goal was determined of the structure, composition, and metabolic profiles of soil microbiomes in various cultivation methods (under multiannual plow and no-plow cultivation) using metagenomic analysis. Having regard to the recommendations contained in EU report (European Commission et al., 2020) of the Mission board for Soil health and food, 2020 indicating the lack of microbiological indicators of "healthy soil". So, we have tried to select of microbiological indicators showing sensitivity and resistance to use the methods of soil cultivation. The research object was located on almost 100-year field experiments at the Experimental Station of the Faculty of Agriculture and Biology in Skierniewice/near Warsaw on, luvisoils dominated in the temperate climate of Central Europe. Soil microorganisms respond with changes in their abundance and taxonomic composition depending on the methods of soil cultivation. *Actinobacteria* were the most abundant, while *Planctomycetes* were the least abundant in the metagenome of soil fertilized with manure, whereas the uncultivated soil was dominated by *Nitrospirae*. We can recommend the following taxa, including *Gemmatimonas* sp. as a microbiological indicator sensitive to the long-term lack of both plow cultivation of soil and organic fertilization, and *Mycobacterium* sp. as a resistancivity indicator to this soil cultivation method. *Sorangium* sp. could be recommended as microbiological indicators which responds by reducing the quantity under effect of the organically fertilized soil, while the plow and no-plow cultivation does not affect changes in its quantity. The use of various cultivation methods changed the biochemical functions in soil metagenoms, including nitrogen and sulfur metabolism and carbohydrate metabolism, and in the production of plant hormones and siderophores. Additionally, soil cultivation ways changed the response of microorganism's stresses, including oxidative stress. The conducted research indicates the necessity to conduct further research on the influence of various cultivation methods, on the diversity of the microorganism community and soil metabolism. The result of which may be the selection of appropriate

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microbiological indicators for determining "soil health" depending on the type of soil under cultivation located in different climatic zones, not only presented in the paper.

1. Introduction

Long-term field experiments are today a unique source of knowledge on the impact of various agricultural systems on the natural environment. Among other things, they play an important role in understanding many changes in the physicochemical and biological properties of the soil. Significant changes in the physicochemical and biological properties of the soil under the influence of varied organic fertilization, mineral fertilization, or crop rotation occur very slowly. Determining the direction of these changes is possible only after a long time – in permanent field experiments. With the WULS-SGGW (Warsaw University of Life Sciences – SGGW) experimental facility in Skierniewice, where different mineral and organomineral fertilization regimes have been applied since 1923 in various crop rotations, attempts can be made to assess the direction of these changes (Mercik et al., 2000; Mercik and Stępień, 2005).

Soil fertility is a resultant of its abiotic (physical, chemical) and biotic properties, which are determined by many factors, including broadly understood agriculture. Nowadays, it is believed that the future system of agriculture is sustainable agriculture, which should simultaneously pursue production, ecological, economic, and social goals. The idea of this system is based on, *inter alia*, minimizing interference with the soil system and diversifying crop rotation. Research shows that long-term diversified crop rotation and fertilization have a more or less beneficial effect on soil fertility. The value of soil environment can also be lowered by prolonged, intensive cultivation. Kuś and Siuta (1999) state that there is no clear effect of various systems of plant succession on soil environment. As reported by Hasinur Rahman et al. (2008) and Martínez et al. (2016), lack of soil cultivation leads to changes in the organic matter content along the soil profile from the surface of the field downwards. These changes have a positive effect on soil physical properties and, as reported by Irfan et al. (2013), as well as Swędrzyńska et al. (2013), on soil biological properties. However, Crawford et al. (2015) and Peterson et al. (2012) showed that definitive changes in soil properties did not occur until about 10 years after the discontinuation of soil cultivation. In addition to mineral fertilization, long-term use of manure also leads to changes in the above-mentioned parameters, including a significant increase in the nitrogen content in the soil environment (Wang et al., 2012; Li et al., 2020: Kołodziejczyk et al., 2017; Zhang et al., 2019).

The soil is inhabited by microorganisms belonging to various taxonomic units and functional groups. The community of bacteria colonizing the Earth's soil ecosystems consists mainly of 12 bacterial phyla, i.e., *Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Verrucomicrobia, Bacteroidetes*, *Gemmatimonadetes, Firmicutes, Armatimonadetes*, TM7 (bacteria of the candidate phylum TM7), and WS2 (candidate division WS2 bacterium) (Delgado-Baquerizo et al., 2018). Dominant among them are only the first four phyla of bacteria, which are very important for the quality of the soil environment (Delgado-Baquerizo et al. 2018). In arable soils, however, the dominant bacterial phyla are *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes,* and *Acidobacteria* (Xu et al., 2009; Bakker et al., 2015). Among *Acidobacteria, Actinobacteria, Planctomycetes, Chloroflexi,* and *Gemmatimonadetes* there are slow-growing bacteria that follow the k-strategy, while *Proteobacteria* (mainly from the classes *Alphaproteobacteria* and *Gammaproteobacteria*) and *Firmicutes* are fast growing and follow the r-strategy (Pascault et al., 2013; Maron et al., 2018).

The fungal communities of each soil consist mainly of taxa in the phylum rank, i.e., *Ascomycota, Basidiomycota* and *Zygomycota* (Yang et al., 2017; Grządziel and Gałązka, 2019).

Agrotechnical procedures carried out to produce crop plants with the best qualitative and quantitative characteristics affect not only the physical and chemical properties of soil ecosystems, but above all their biological, including microbiological properties (Nam et al., 2021; Bhattacharyya et al., 2022).

Agricultural management is thus a way of affecting soil microbiomes to improve soil quality and improve plant production and soil services without harming the environment. Integrating knowledge on soil microbial communities with other levels of the soil food web is the future of soil science and could also be one of the hints to help mitigate global climate changes (United Nations Framework Convention on Climate Change, 2011; World Meteorological Organization, 2019).

In recent years, there has been a significant development of metagenomic technologies, which have been used in Microbiology for comprehensive analysis of not only *in vitro* culturable (1–3%), but above all non-culturable (99–97%) microorganisms. As a result, it is possible to determine the structure, taxonomic composition, and metabolism of a soil microbial community, which can then be used to estimate indicators of the general quality status of a soil and its production potential (Torsvik et al., 2002; Djokic et al., 2010). With reference to the EU report (European Commission et al., 2020) of the Mission board for "Soil health and food" it is necessary to undertake research in order to complete the knowledge of microbiological indicators of "healthy soil". As highlighted in this document, studies are necessary to take into account the different soil use according to climate, because it cannot be generalized. Therefore, the soil type predominant in central Europe, the luvisoils, was chosen for the study.

The aim of the presented studies was to determine the structure of the microorganism community, including prokaryotes and eukaryotes, in a soil classified as luvisoils – dominated in the temperate climate. Subjected to many years of plough and ploughless tillage, and to determine the effects of organic fertilization or its absence. Next, the relationship between the use of soil tillage methods and the dominant taxa of microorganisms in the soil was determined. In addition, microbiological indicators showing sensitivity and resistance to perennial plow and no-plow cultivation of soil were selected for use as indicators to determine the quality of luvisoils soil, including "healthy soil" in temperate climates.

2. Methods

2.1. Site location and sample collection

The research was conducted at the Experimental Station of the Faculty of Agriculture and Biology in Skierniewice near Warsaw (Lat: 51°58′N; Long: 20°10′E) on a soil classified as luvisols. The humus horizon (Ap) of this soil, with a thickness of approx. 25 cm, is made up of heavy loamy sand (16% of $<$ 0.02 mm fraction), and the Et (eluvial leaching) wash-out horizon (26–45 cm) of slightly loamy sand (13% of $<$ 0.02 mm fraction). The Bt (iluvial accumulation) wash-in horizon (46–75 cm) and bedrock below 75 cm are made up of light clay (25–30% of $<$ 0.02 mm fraction). The climatic conditions for this region are typical of central Poland. Based on the average results of measurements from the period 1921–2019, the average annual air temperature was 8.1 °C, average annual rainfall – 536.1 mm, average annual exposure to sunlight – 1707 h, average annual air humidity – 79%, and the average annual duration of the growing season (temperature $>$ 5 °C) – 213 days.

The soil for analysis was collected from the arable layer after crop plants had been harvested in the autumn of September 2019, from three fields of long-term fertilization experiments conducted since 1923 by the Department of Agricultural Chemistry of the Warsaw University of Life Sciences in Skierniewice. The long-term fertilization experiments had been established in 3 replications in a randomized block design, in 6

fertilization combinations: CaNPK (calcium, nitrogen, phosphorus, potassium), NPK (nitrogen, phosphorus, potassium), PK (phosphorus, potassium), NP (nitrogen, phosphorus), NK (nitrogen, potassium), and 0. For the analyses described in this article, soil samples were taken from the arable layer of the CaNPK combination, 15 individual samples from each replicate (36 m^2 plots), according to PN-ISO 10381 (Soil quality – Sampling – Part 1: Guidance on the design of sampling programmes). After collection, the samples were prepared for analysis in accordance with ISO 11465 (Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method). The soil was sampled in the fourth year after liming and after fertilization with manure from fields representing the following three soil cultivation methods (experimental variants):

- No. 1 – plow tillage with mineral fertilization (CaNPK)
- No. 2 – plow tillage, fertilization with CaNPK + manure at 25 t/ha every 4 years
- No.3 – no tillage since 1975 (highbush blueberry plantation), mineral fertilization with CaNPK

Fresh cattle manure from a shallow barn was used in the experiments.

Soil samples were taken with an Egner's auger, 10 punctures for each of 4 replication plots avoiding the borders, from which a pooled sample was prepared from each agriculture management system, i.e., about 300 g of soil. Part of the soil material was frozen until further analysis for DNA (deoxyribonucleic acid).

2.2. Soil analysis

Selected physico-chemical properties were determined in the soil samples, including pH (quantitative scale of alkalinity and acidity of an aqueous solution) in 1 M KCl (potassium chloride); total carbon by the direct method according to PN-EN 15936 (Sewage sludge, treated biowaste, soil and waste – Determination of total organic carbon (TOC) after dry combustion); total nitrogen by the Kjeldahl method; available phosphorus by the Egner-Riehm method; exchangeable cations (K, Ca, Mg, Na) in 1 M CH_3COONH_4 (ammonium acetate) by the Jackson method; soil water capacity using a ring infiltrometer according to PN-ISO 11461 (Soil quality – determination of soil water content, expressed as a volume fraction, using the ring method – weight method).

2.3. DNA extraction, PCR, taxonomic and functional insight

Metagenomic DNA was isolated using a modified method based on the Genomic Mini AX Bacteria + kit (A&A Biotechnology, Gdynia, Poland). Additional mechanical lysis of samples was performed with zirconia beads in a FastPrep-24 homogenizer. The presence of bacterial DNA in the test samples was confirmed by Real-Time PCR (Polymeraze Chain Reaction). RT-PCR reactions were performed in an Mx3000P thermocycler (Stratagene) using SYBR® Green dye as fluorochrome. Universal primers were used in the reactions (Ferris et al., 1996).

Composition of the reaction mixture: Real time 2x-PCR Mix SYBRA (A&A Biotechnology) – 10 μl; primer 1055F 10 μM (5′-ATGGCTGTCGTCAGCT-3′) – 0.4 μl; primer 1392R – 10 μM (5′ACGGGCGGTGTGTAC-3′) – 0.4 μl; DNA – 0.2 μl; water – make up to 20 μl.

Temperature-time profile of the reaction: initial denaturation (95 °C, 3 min), denaturation (95 °C, 15 s), primer annealing (58 °C, 30 s), fluorescence reading, PCR product extension (72 °C, 30 s), determination of melting curve of PCR product by measuring fluorescence at each temperature (from 65 °C to > 95 °C).

After isolation, metagenomic DNA (250 ng) was fragmented using a Covaris E210 instrument into fragments ranging in length from 200 to 500 bp. AMPure XP Beads (Beckman Coulter) was used for purification. Libraries were prepared using the NEBNext® DNA Library Prep Master Mix Set for Illumina (New England Biolabs) according to the manufacturer's instructions. The libraries were checked with a 2100 Bioanalyzer using High-Sensitivity (Agilent) chips and quantified by qPCR (quantitative polymerase chain reaction). Sequencing took place on one lane of a HiSeq4000 (Illumina) sequencer.

Demultiplexing of the samples was performed with the bcl2fastq2 program (v2.20.0.422). Adapter sequences were cut off with cut adapt (v1.18). To unravel the microbial and functional diversity of the samples *in silico*, metagenomic sequences were examined with the Metagenomic Rapid Annotations using Subsystem Technology (MG-RAST) online server (Meyer et al., 2008).

2.4. Statistical data analysis

To determine the differences in the relative abundance of each taxon between the soil cultivation treatments, the results were verified by the univariate analysis of variance; homogeneous groups were identified with the Tukey test for $\alpha \leq 0.05$. Principal component analysis (PCA) was performed to emphasize variation and show patterns in the metagenome datasets.

3. Results

3.1. Soil characteristics

The soil systematically fertilized with manure (No. 2) was characterized by the highest levels of organic carbon, nitrogen, magnesium, and potassium. Manure also improved the water capacity of the soil (Table 1). The worst physico-chemical properties for all the parameters tested were found in the soil that had not been fertilized with organic fertilizers for almost 100 years (No. 1). The lack of tillage since 1975 had limited the extent of soil degradation. This had a particularly positive effect on soil pH and the levels of calcium and phosphorus. The amounts of these elements in that soil (No. 3) were even higher than in the soil systematically fertilized with manure (No. 2).

Results from principal-component analysis (PCA) of the soil samples with respect to the soil properties allowed to observe a clear separation between soil variants along the PC1 axis (Fig. 1). A strong positive correlation was evident between such traits as: N, Na, Mg, C, K, and capillary water capacity, which were the highest in variant No. 2 with manure, and the lowest in variant No. 1 – without manure. A strong positive correlation was also found between Ca, pH, and P, with the highest values for variant No. 3 – under no plow cultivation.

3.2. Microbial diversity and taxonomic composition

In the tested soils, at *Proteobacteria* and *Actinobacteria* phyla were the most abundant (Table 2). However, there were not statistical differences in the relative abundance of *Proteobacteria, Acidobacteria, Bacteroidetes, Firmicutes* in the soil treatments. The highest percentage of *Actinobacteria* was found in the soil fertilized with manure (soil variant 2). Similar abundance of *Planctomycetes* was found in the soil not fertilized with a natural fertilizer (soil variants 1 and 3). The phylum *Verrucomicrobia* was also most abundant not fertilized soils 1 and 3, while the *Gemmatimonadetes* and *Chloroflexi* had a similar abundance in plowed soils (1 and 2).

Statistical differences ($p < 0.05$) were also observed at order level (Fig. 2). Reads affiliated with *Actinomycetales* and *Sphingomonadales* were more abundant in plowed soil with manure and higher abundance of reads affiliated to *Rhizobiales* were found in no plowed soil. (Fig. 2). *Nostocales* and *Burkholderiales* were higher in variant 1, while *Desulfuromonadales* and *Xanthomonadales* were higher in variant 3. In contrast, less abundances of *Planctomycetales* and *rhizobiales* were found in soil variant 2.

The PCA analysis showed that the content of Ca, P and pH was associated with no plowed soil (soil variant 3) and the taxa

Table 1
Physico-chemical properties of soil under three methods of soil cultivation [± SD (n = 3)].

Cultivation method	C [% in DW]	N [% in DW]	pH	P [mg·kg^{-1}]	K [mg·kg^{-1}]	Ca [mg·kg^{-1}]	Mg [mg·kg^{-1}]	Na [mg·kg^{-1}]	capillary water capacity [m^3·ha^{-1}]
No.1 without manure	0.66^a	0.037^a	5.40^a	47.87^a	49.33^a	55.67^a	42.33^a	15.03^a	732.0^a
No.2 with manure	0.77^c	0.045^c	5.60^a	52.83^b	59.53^c	62.50^b	60.47^c	16.63^c	755.7^c
No.3 under blueberry	0.70^b	0.042^b	5.97^b	67.47^c	53.43^b	66.97^c	49.17^b	15.90^b	739.7^b
LSD α = 0.05	0.0400	0.0025	0.3011	2.6631	2.0911	2.8220	2.1356	0.4497	6.2492

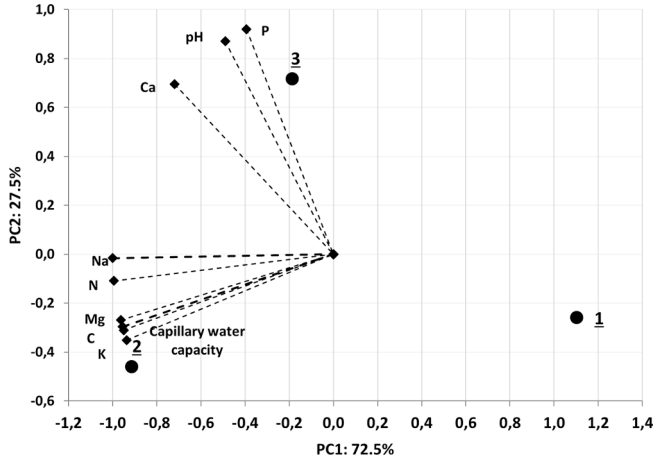

Fig. 1. Principal component analysis (PCA) of the soil variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation and the soil properties. PCA was calculated using means, which were calculated using 3 replications.

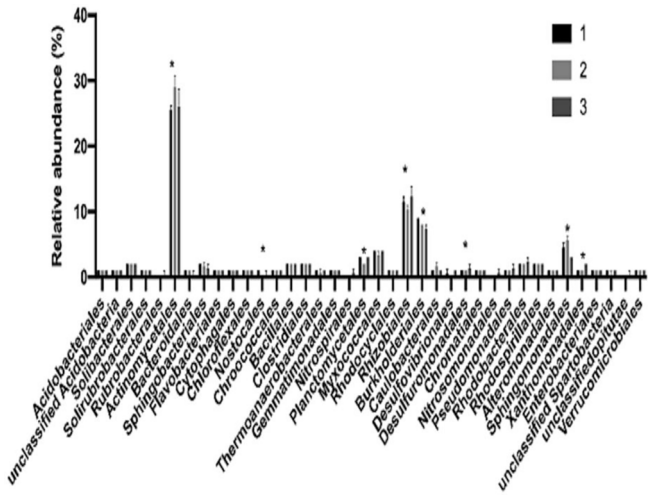

Fig. 2. Relative abundance of bacterial sequences at order level from soil metagenomes variants: 1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation based on MG-RAST affiliations of unassembled Illumina reads of soil variants.

Table 2
Dominant phyla of microorganisms (relative abundance ≥ 0.36% in at least one of the samples) and the difference analysis distributed in the three experimental variant soils, n = 3 per each experimental variant of soil. The Tukey test was used to assess the significance between the soils, α ≤ 0,05.

Phylum	1	2	3	LSD
Proteobacteria	45.84 ^{a}	41.97 ^{a}	45.35 ^{a}	5.0485
Actinobacteria	29.49 ^{a}	35.26^b	28.83 ^{a}	5.5430
Acidobacteria	4.34 ^{a}	4.79 ^{a}	3.93 ^{a}	1.7662
Bacteroidetes	4.26 ^{a}	4.35 ^{a}	3.22 ^{a}	2.1048
Planctomycetes	2.78^b	1.9 ^{a}	2.90^b	0.7243
Verrucomicrobia	2.48 ^{ab}	1.81 ^{a}	2.77^b	0.7580
Chloroflexi	2.18^b	1.86 ^{ab}	1.47 ^{a}	0.5263
Firmicutes	2.0 ^{a}	1.96 ^{a}	1.96 ^{a}	0.3950
Gemmatimonadetes	1.77^b	1.93^b	1.04 ^{a}	0.2738
Cyanobacteria	1.09^b	0.80 ^{a}	0.90 ^{ab}	0.2551
Nitrospirae	0.46 ^{a}	0.37 ^{a}	0.76^b	0,0950

Solirubrobacterales, *Xanthomonadales*, *Verrucomicrobiales* and *Rhizobiales* (Fig. 3). In addition, the content of N, Na, Mg, K, C and capillary water capacity were positively related with *Actinomycetales* and soil plowed and fertilization with manure.

Strong positive correlations were found between the abundances of microorganisms at the rank of family, i.e., *Solibacteraceae, Sphingomonadaceae, Gemmatimonadaceae* and *Comamonadaceae*, which were most abundant in the soil of the cultivation variant No. 2 (Fig. 4). At the same time, their abundances were negatively correlated with *Mycobacteriaceae, Burkholderiaceae* and *Bradyrhizobiaceae*, which had the highest % share in the soil of variant No. 3. A positive strong correlation was also found between *Nocardioidaceae* and *Conexibacteraceae*, which were least abundant in soil No. 1 (Table 3).

The percentage of actinomycetes of the genus *Streptomyces* spp. in the

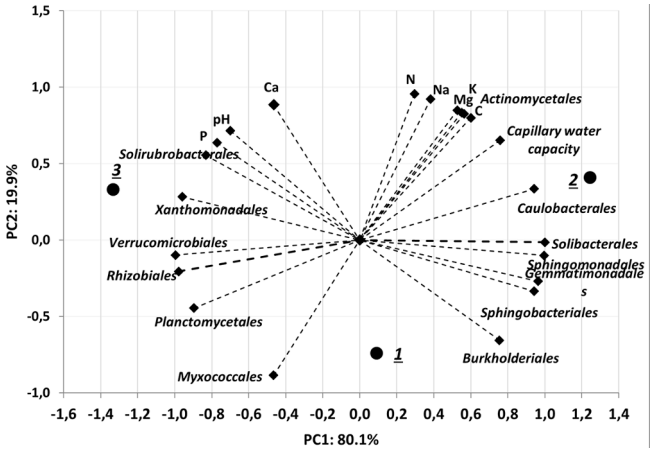

Fig. 3. Principal Component Analysis (PCA) of relative abundances of microbial taxa at order level and the relation with the three soil experimental variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation] and properties. PCA was calculated using means, which were calculated using 3 replications.

soil that had not been tilled nor fertilized with manure for many years (No. 3) was comparable with that in the soils under plough tillage and fertilized or not with manure (No. 2 and No. 1). By comparison, the soil under long-term tillage and fertilized with manure, on which different crops had been grown (No. 2), had a higher % share of *Streptomyces* spp. and *Gemmatimonas* spp. than the soil similarly tilled but without manure fertilization (No. 1). In soil No. 3, there was a significantly higher

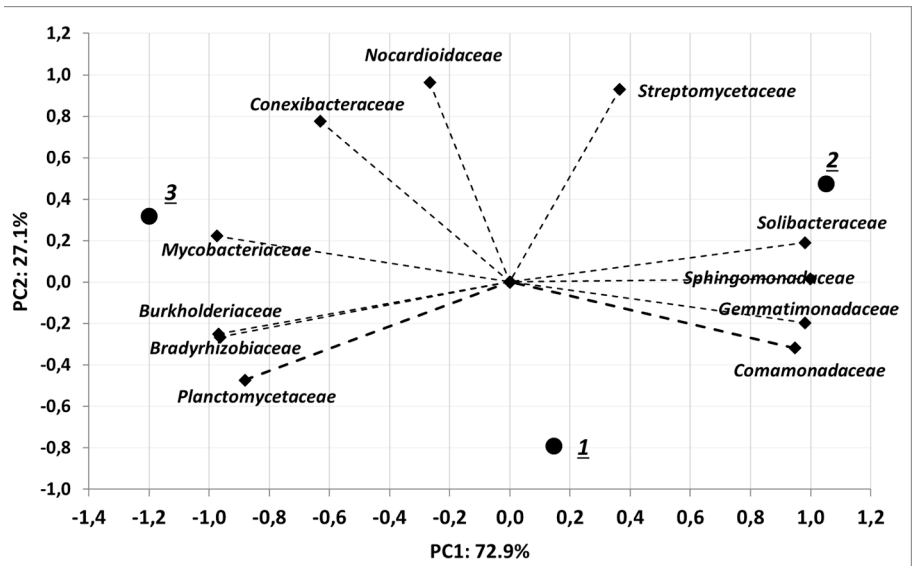

Fig. 4. Principal component analysis (PCA) of relative abundances of microbial families and the statistically significant relation with three soil experimental variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation].

Table 3
Dominant genera of bacteria (relative abundance ≥ 1% in at least one of the samples) and the difference analysis distributed in the three experimental variant soils, n = 3 per each soil sample. The Tukey test was used to assess the significance between soils, α ≤ 0,05.

Genus	Variant I (%)	Variant II (%)	Variant III (%)	LSD
Candidatus Solibacter	4.22 [a]	4.77 [a]	3.08 [a]	1.3755
Streptomyces	4.18 [a]	5.02[b]	4.51 [ab]	0.7543
Conexibacter	3.49 [a]	3.77 [a]	4.06 [a]	0.9192
Gemmatimonas	3.47 [a]	3.91[b]	1.8 [a]	0.4272
Nocardioides	3.23 [a]	4.41 [a]	4.89 [a]	4.6908
Mycobacterium	2.86 [a]	2.59 [a]	3.98[b]	0.9695
Sorangium	2.56[b]	1.64 [a]	2.35[b]	0.6421
Bradyrhizobium	2.09 [a]	1.81[a]	1.93 [a]	0.4865
Frankia	1.63 [a]	1.90 [a]	1.88 [a]	0.3920
Burkholderia	1.76 [a]	1.71 [a]	1.84 [a]	0.4622

percentage of *Mycobacterium* spp. in comparison with the other experimental variants (Nos. 1 and 2).

The abundances of the taxa of *Mycobacterium* and *Burkholderia* were highly positively correlated with each other and were most abundant in the soil under blueberry (No. 3) (Fig. 5). At the same time, these traits were negatively correlated with *Candidatus Solibacter* and *Gemmatimonas*, which were most abundant in the soil of variant No. 2.

A strong positive correlation was also found between *Sorangium* and *Bradyrhizobium*, which had the highest values for the soil from variant No. 1. On the basis chang in the abundance of genera including *Gemmatimonas* sp., *Mycobacterium* sp.,and *Sorangium* sp. depends on the used of soil cultivation methods this bacteria can be recommended as microbiological indexes. *Gemmatimonas* sp. can be recommended as microbiological indexes sensitive to lack of both plow cultivation of soil and aplication of manure, because it respond by decreasing in its aboundence about 50% in compered to other experimental variants). While *Mycobacterium* sp. is recommended as resistancivity index on the long-term lack of plow cultivation as well as organic fertilization (respond by incresing its aboundence about 30% in compered to No.1 and No2 variants (Table 4).

On the other hand, *Sorangium* sp. is very sensitive to the use of manure, as its number statistically significantly decreased by about 35% compared to both plow and no-plow tillage (Fig. 6).

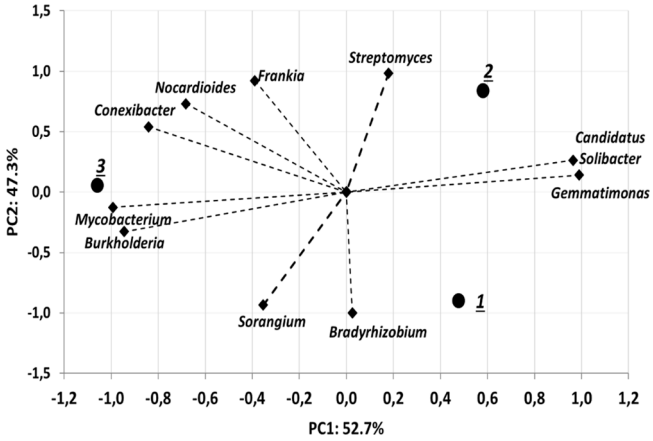

Fig. 5. Results of principal component analysis (PCA) of relative abundances of bacterial genera and the statistically significant relation with three soil experimental variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation].

The metagenomic analyses *in silico* showed a slight % share of dominant fungi in the soils of all the fertilization variants (No. 1, 2, 3). However, in the soil fertilized with manure (No. 2), this percentage was higher (not always statistically significant) than in the tilled/untilled soils not fertilized with manure (No. 1 and No. 3). In soil No. 2, the most abundant fungi were those of the order *Sordariomycetes* and *Eurotiomycetes* (Table 4). Most of the fungal taxa at the rank of genus were quite closely correlated with one another. In particular, strong positive correlations were found between the abundances of *Talaromyces* sp., *Aspergillus* sp., *Pyrenophora* sp., *Penicillium* sp., *Nectria* sp., *Emericella* sp., and *Neosartorya* spp. Soils No. 1 and 3 were characterized by a much lower % share of almost all the fungal taxa listed (Fig. 7).

3.3. Functional profile of the arable soil microbiome

The main SEED (functional annotation) subsystems level 1 comprised 28 major metabolic classes (Table 5), the most abundant annotated reads belonged to clustering-based subsystems (CBSS), carbohydrate metabolism, and amino acids and derivatives (Table 5). Some metabolic categories were less abundant such as dormancy and

Table 4
Dominant genera of Fungi (relative abundance ≥ 0,01% in at least one of the samples) and the difference analysis distributed in the three experimental variant soils, n = 3 per each soil sample. The Tukey test was used to assess the significance between soils, α ≤ 0,05.

Phyllum/class	Genus	Variant I	Variant II	Variant III	LSD
Ascomycota/Eurotiomycetes	*Aspergillus*	0.032[a]	0.044[b]	0.033[a]	0,0084
	Coccidioides	0.011[a]	0.015[b]	0.010[a]	0.0038
	Emericella	0.011[a]	0.015[b]	0.011[a]	0.0038
	Neosartorya	0.042[a]	0.061[b]	0.042[a]	0.0158
	Penicillium	0.017[a]	0.029[b]	0.018[a]	0.0075
	Talaromyces	0.009[a]	0.016[b]	0.010[a]	0.0053
Ascomycota/Sordariomycetes	*Chaetomium*	0.016[a]	0.019[b]	0.012[a]	0.0055
	Gibberella	0.042[a]	0.048[a]	0.040[a]	0.0082
	Magnaporthe	0.017[a]	0.019[b]	0.016[a]	0.0082
	Nectria	0.018[a]	0.020[b]	0.018[a]	0.0052
	Neurospora	0.025[a]	0.029[b]	0.021[a]	0.0053
	Podospora	0.015[a]	0.018[b]	0.012[a]	0.0029
Ascomycota/Leotiomycetes	*Botryotinia*	0.009[a]	0.011[a]	0.010[a]	0.0045
	Sclerotinia	0.011[a]	0.013[b]	0.010[a]	0.0045
Ascomycota/Dothideomycetes	*Phaeosphaeria*	0.023[a]	0.029[b]	0.022[a]	0.0049
	Pyrenophora	0.011[a]	0.016[b]	0.012[a]	0.0043
Ascomycota/Tremellomycetes	*Filobasidiella*	0.018[a]	0.023[b]	0.017[a]	0.0045
Ascomycota/Schizosaccharomycetes	*Schizosaccharomyces*	0.012[a]	0.013[a]	0.013[a]	0.0035
unclassified (derived from Eukaryota)	*Phytophthora*	0.013[a]	0.013[a]	0.012[a]	0.0057
Basidiomycota/Ustilaginomycetes	*Ustilago*	0.011[a]	0.012[a]	0.012[a]	0.0045

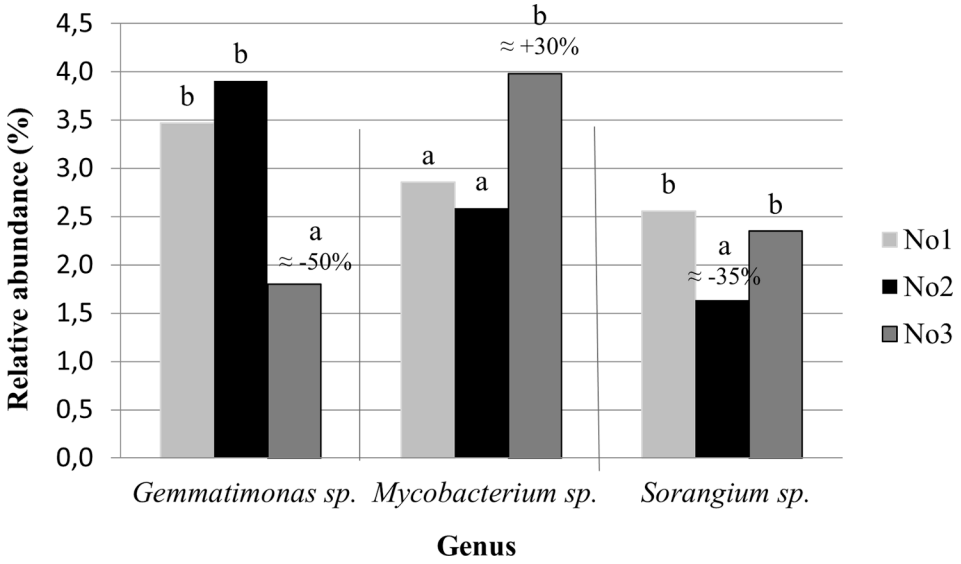

Fig. 6. Genus ranked bacterial taxons in advised as sensitivity and resistance indexes on both the plow cultivation with and without FYM (farmyard manure) (No. 1 and No. 2) as well as no-plow cultivation (No.3).

sporulation and potassium metabolism.

In order to make a comparison between the different methods of soil tillage, a principal component analysis was generated based on the distribution of 28 major metabolic sub-system classes (Fig. 8). It can be seen that the abundance of some metabolic groups is related to a specific method of soil cultivation.

In the metagenome of soil No. 1, where long-term plow tillage had been applied without organic fertilization, and on which various annual plants had been grown, the most abundant and highly positively correlated gene sequences were those responsible for: Membrane Transport, Regulation and Cell signalling, Dormancy and Sporulation, Photosynthesis, Iron acquisition and metabolism, and Cell Wall and Capsule. Similar correlations, but for Secondary Metabolism, Amino Acids and Derivatives, Nitrogen Metabolism, Nucleosides and Nucleotides were found for the no ploughed soil, which had not been subjected to long-term plough tillage nor fertilization with manure (soil No. 3). At the same time, the listed metabolic groups of microorganisms were negatively correlated with sequences related to the metabolism of Fatty Acids, Lipids and Isoprenoids, DNA Metabolism and RNA Metabolism, Carbohydrates, Protein Metabolism, Cofactors, Vitamins, Prosthetic Groups, Pigments, and Cell Division and Cell Cycle, which were most abundant in the soil subjected to long-term plough tillage and fertilized with manure, on which various annual plants had been grown (soil No. 2).

Interesting statistical differences were observed in nitrogen and sulphur metabolism and stress response. Inside the nitrogen metabolism category, the functions associated with assimilation of ammonia (statistically significant) and nitric oxide synthase (no statistically significant differences) were higher in soil with no plow (variant 3). While higher abundance of genes associated nitrate and nitrite assimilation and denitrification, but no statistically significant differences were found in plow soils with and without applied of manure (variant 1) (Fig. 9A). In the category of stress response, the most abundant metabolic type in this category was the oxidative stress followed by osmotic stress, which were higher (no statistically significant differences) in ploughing soils with and without manure (variant 1 and 2) (Fig. 9B). In

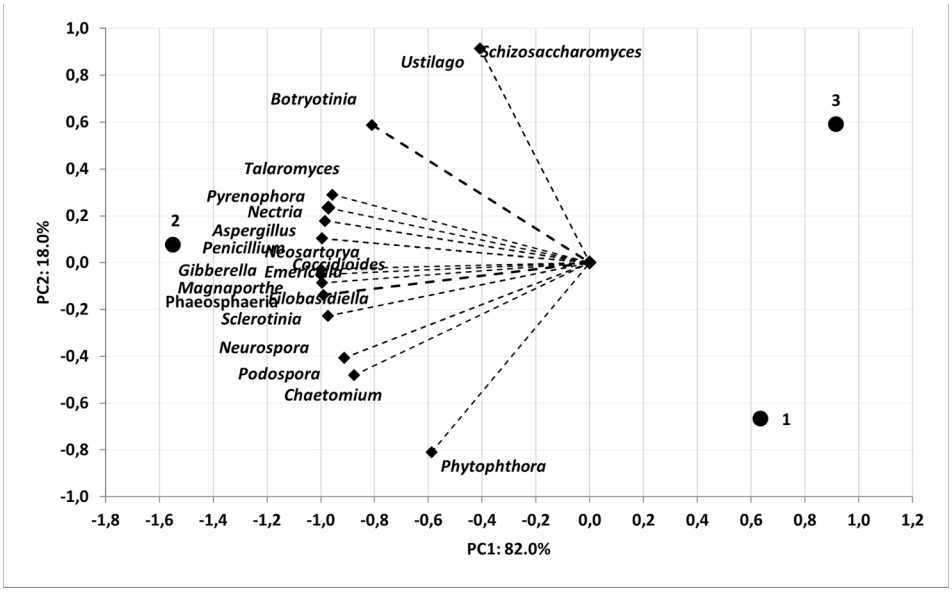

Fig. 7. Principal component analysis (PCA) of relative abundances of fungal genera and the relation with three soil experimental variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation].

the category of sulphur metabolism, the relative abundance of inorganic and organic sulphur assimilation was higher in soil variant 1 (no statistically significant differences) (Fig. 9D).

Inside carbohydrates category, the subsystems related to central carbohydrate metabolism, monosaccharides and fermentation were the most represented (no statistically significant differences) in the datasets and specially in higher abundance for soil variants 1 and 2 (Fig. 9C). In addition, similar abundances of genes associated with biosynthesis of auxins were observed in three experimental variants (no statistically significant differences) (Fig. 9E) but higher abundance of siderophore (no statistically significant differences) related sequences were found in soil variant 1 (Fig. 9F).

4. Discussion

In modern agriculture in the EU, organic fertilization is often abandoned, and the soil cultivation system limited. The influence of various tillage and fertilization systems on the physical, chemical, and especially microbiological properties of the soil is noticeable only after several years. Therefore, with an experimental facility where long-term experiments had been conducted in a static system for many years, research was undertaken on the response of soil microbial communities and their potential functional properties in the soil where organic fertilization or no such fertilization, and plough tillage or no tillage, had been applied. Of particular interest was the assessment of the extent to which the changes in soil physico-chemical properties affect microbial activity.

The main goal of spatial development of agro ecosystems should be for agriculture to adopt reasonable strategies to prevent negative effects on the quality of the environment, including the soil as one of its components.

The research undertaken in this study shows the response of communities of soil microorganisms and their potential functional properties in the soil of long-term fertilization experiments where varied mineral and organic fertilization and different soil cultivation techniques (plough tillage and no-tillage) had been applied.

Additional organic fertilization applied every 4 years over 45 years as compared with the soil not fertilized with an organic fertilizer since 1923 had significantly improved all the tested physical and chemical properties of the soil (Table 1). The levels of magnesium, organic carbon, and nitrogen had increased the most, and the soil pH and calcium content the least. Beneficial effects of organic fertilization on soil properties have been reported by many authors (Sollins et al., 1996; Steiner et al., 2007; Mueller et al., 2012; Bowles et al., 2014; Bhattacharyya et al., 2022).

The soil under the perennial plantation of highbush blueberry (since 1975) was less prone to degradation than the soil under full tillage. The lack of tillage for several decades had resulted in less leaching of nutrients. In addition, the uptake of nutrients by blueberry plants (taken away with the crop) is several times lower than is the case with agricultural plants. Therefore, in the soil under blueberry (No.3), higher values of all the tested physico-chemical parameters were obtained than in the soil subjected each year to full tillage (No.1). Particularly noteworthy is the higher level of organic carbon, which indicates a lower degree of mineralization and a greater water capacity and is thus evidence of a better soil structure under blueberry compared with cultivation based on plough tillage (Prakash et al., 2010; Johnston and Poultron, 2018). The increase in carbon content in untilled soil has been pointed out by Małecka et al. (2012), the improvement in soil structure by Wacławowicz et al. (2012), and the improvement in water capacity by Lepiarczyk et al. (2007).

The soil cultivation technique had not significantly affected the relative abundances of *Protobacteria*, *Acidobacteria*, *Bacteroidetes, Firmicutes* as major phyla in the soils (No. 1, 2, 3). Regardless of whether plow tillage was used (Nos. 1, 2) or not (No. 3), the dominant taxa in the soils were *Proteobacteria* and *Actinobacteria*, what is agree with results presented in "A global atlas of the dominant bacteria found in soil" (Delgado-Baquerizo et al., 2018). The highest percentage of *Actinobacteria* was found in the soil fertilized with manure (No. 2), what is due to the good chemical properties of that soil (the highest levels of C, N, K, Mg, Na). Which is beneficial to the properties of this soil as *Actinomycetes* are the main producers of extracellular enzymes and secondary metabolites in soil ecosystems and are also believed to contribute significantly to the carbon cycle, suppressing plant disease, and promoting plant growth.

The *Verrucomicrobia*, characteristic of oligotrophic soils, and the *Planctomycetes*, of inland reservoirs and soil ecosystems, colonized in the largest numbers the tilled/untilled soils, but not fertilized with manure (No. 1 and No. 3). *Verrucomicrobia* are oligotrophs oxidation of methanol in the soil which is a more important process because methanol likely is a significant source of labile organic carbon to microorganisms inhabits in the soil ecosystems. The methanol fluxes likely representing an important, but under-studied mechanism, by which organic carbon is transferred from both surface litter to mineral layer of the soil (Fierer,

Table 5
Functional composition of the soil metagenomes. Relative distribution (in percentage of annotated reads) of 28 major metabolic subsystems (using SEED subsystems in the MG-RAST program) detected in the soil metagenomes of the experimental variants. The Tukey test was used to assess the significance between soils for each metabolic class, $\alpha \leq 0{,}05$.

No.	Metabolic Class	Variant 1 [%]	Variant 2 [%]	Variant 3 [%]	LSD
1	Carbohydrates	15.07 ^{a}	15.15 ^{a}	14.93 ^{a}	0.255
2	Clustering based subsystems*(CBSS)	13.72 ^{a}	13.78 ^{ab}	13.81 ^{b}	0.067
3	Amino Acids and Derivatives	11.49 ^{ab}	11.46 ^{a}	11.55 ^{b}	0.0699
4	Miscellaneous	7.24 ^{a}	7.22 ^{a}	7.25 ^{a}	0.0236
5	Protein Metabolism	6.89 ^{a}	6.92 ^{a}	6.89 ^{a}	0.122
6	Cofactors, Vitamins, Prosthetic Groups, Pigments	6.57 ^{a}	6.67 ^{b}	6.60 ^{ab}	0.081
7	RNA Metabolism	4.67 ^{a}	4.69 ^{a}	4.65 ^{a}	0.087
8	Fatty Acids, Lipids, and Isoprenoids	4.66 ^{a}	4.69 ^{a}	4.65 ^{a}	0.08678
9	DNA Metabolism	3.76 ^{ab}	3.84 ^{b}	3.73 ^{a}	0.103
10	Cell Wall and Capsule	3.70 ^{a}	3.66 ^{a}	3.67 ^{a}	0.1002
11	Respiration	2.70 ^{a}	2.70 ^{a}	2.72 ^{a}	0.134
12	Virulence, Disease and Defense	2.56 ^{a}	2.49 ^{a}	2.56 ^{a}	0.083
13	Nucleosides and Nucleotides	2.45 ^{a}	2.45 ^{a}	2.46 ^{a}	0.3125
14	Stress Response	2.23 ^{a}	2.21 ^{a}	2.25 ^{b}	0.0205
15	Membrane Transport	2.21 ^{b}	2.12 ^{a}	2.15 ^{ab}	0.075
16	Metabolism of Aromatic Compounds	2.0 ^{a}	1.99 ^{a}	1.98 ^{a}	0.054
17	Regulation and Cell signaling	1.17 ^{b}	1.14 ^{a}	1.14 ^{a}	0.0187
18	Cell Division and Cell Cycle	1.05 ^{a}	1.08 ^{a}	1.06 ^{a}	0.028
19	Sulfur Metabolism	1.04 a	1.00 ^{a}	1.09 ^{b}	0.038
20	Phosphorus Metabolism	1.04 ^{b}	1.01 ^{a}	1.04 ^{b}	0.0251
21	Phages, Prophages, Transposable elements, Plasmids	0.93 ^{a}	0.94 ^{ab}	0.98 ^{b}	0.040
22	Nitrogen Metabolism	0.93 ^{a}	0.94 ^{ab}	0.98 ^{b}	0.040
23	Motility and Chemotaxis	0.74 ^{b}	0.73 ^{ab}	0.72 ^{a}	0.014
24	Iron acquisition and metabolism	0.57 ^{a}	0.53 ^{a}	0.53 ^{a}	0.0723
25	Secondary Metabolism	0.36 ^{ab}	0.35 ^{a}	0.39 ^{b}	0.0251
26	Potassium metabolism	0.32 ^{a}	0.31 ^{a}	0.32 ^{a}	0.017
27	Dormancy and Sporulation	0.10 ^{a}	0.10 ^{a}	0.10 ^{a}	0.009
28	Photosynthesis	0.10 ^{a}	0.10 ^{a}	0.10 ^{a}	0.009

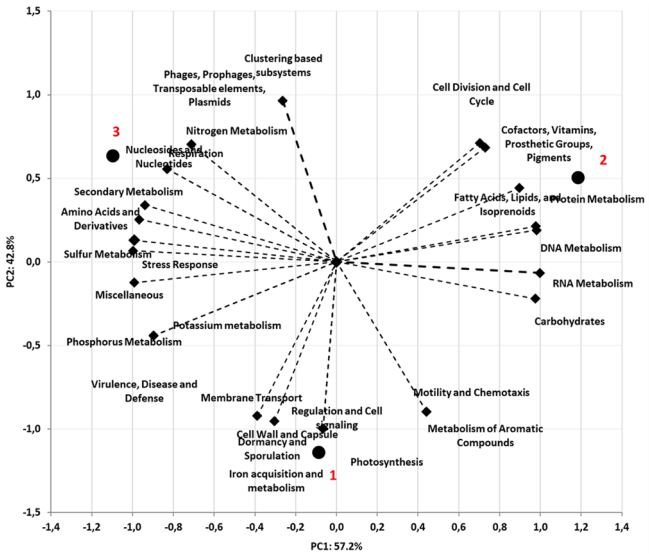

Fig. 8. Principal component analysis (PCA) of relative distribution (in percentage of annotated reads) of 28 major metabolic subsystems and the relation with three soil experimental variants [1- plow cultivation; 2- plow cultivation with manure; 3- no plow cultivation].

2015). These taxa play also important function in carbon cycle thanks degradation of various polysaccharide (Martinez-Garcia et al., 2012). The *Gemmatimonadetes* had a similar % share and abundantly occurrence in the plow tillage soils (Nos.1 and 2), which is confirmed by the test results of Naomi (2013). She has shown that the distribution of *Gemmatimonadetes* is higher in arable soils, and it was more dependent on moisture availability, while members of this phylum are well adapted to low moisture conditions but unable to resist moisture fluctuations in the soil.

The *Chloroflexi* had a similar % share in the non-fertilized soil No. 1 and the manure-fertilized soil (No. 2), where plough tillage had been used for many years. While occurrence of *Chloroflexi* was significantly higher in No. 1 experimental variant compering in no-tillage soil where had not applied manure (No. 3). Aerobic heterotrophic *Chloroflexi* colonize mainly toxic environments lead to they are numerous in plow-tillage soils (Nos.1,2) where there are better oxygen conditions then in no-tillage soils (No.3) (Islam et al., 2019).

The soil under blueberry (No. 3) as well as with plow-tillage (No.1) was abundantly colonized by representatives of *Planctomycetes* which can break down polysaccharides in the soil (Ivanova et al., 2016). However, in these soils (No. 1 and No. 3) not found significant differences in abundance of *Verrucomicrobia* and *Cyanobacteria* what relates to chemical properties of soils, including smaller amount of organic carbon and nitrogen (Table 1.). Compared to the fertilized soil No.2, *Cyanobacteria* abundance was higher in the unfertilized soils, suggesting that the low N content in the unfertilized soil may stimulate the growth of N-fixing *Cyanobacteria*. Similarly, soil No. 3, not fertilized with manure and not tilled for many years, was dominated by oligotrophic bacteria of the phylum *Verrucomicrobia*, which prefer to live in an environment with low nutrient availability (Fierer and Jackson, 2006).

The lack of plow- tillage and annual manure application, and cultivation of blueberry (No.3) resulted in the significantly higher occurrence of *Nitrospirae* (oxidation of nitrites to nitrates) which is corresponding in the highest value of pH, total nitrogen, phosphorous and calcium in this soil compering in the other (Nos.1,2), what is agree with studies of Fan et al. (2020). In addition, since *Nitrospira*-like bacteria are K strategies that prefer lower concentrations of nitrite and oxygen in the environment (Daims et al., 2001; Blackburne et al., 2007; Nowka et al., 2015) so the lack of plowing positively influenced the occurrence of this taxon in soil No.3.

The statistical analysis showed significant differences in the occurrence of a few bacterial order level including *Actinomycetales, Nostocales, Planctomycetales, Rhizobiales, Burkholderiales, Desulfuromonadales, Sphingomonadales* and *Xanthomonadales* dependce on method of soil cultivation as well as chemical properties of the experimental variants of soil (Figs. 2, 3).The highest amount of phosphorus, calcium and pH in the soil of the experimental variant No.3 lead to abundantly occurrences of *Rhizobiales* as well as *Xanthomonadales* (Fig. 3) which is consistent with the results of the studies by Beck and Munns (1985).

Proteobacteria there are microorganisms showing considerable metabolic diversity, including aerobes, anaerobes, heterotrophs, autotrophs, however among of them are plant pathogens, e.g., *Pseudomonas syringe*, *Xanthomonas* spp., and others.

Long-term fertilization with manure and the use of plow tillage on soil No. 2 had increased the relative abundance of potential bacteriopathogens of the family *Sphingomonadaceae*, which are mainly found in “diseased soils” (Buonaurio et al., 2002; Sanguin et al., 2009;). Despite those pathogens, soil No. 2 was also dominated by the plant

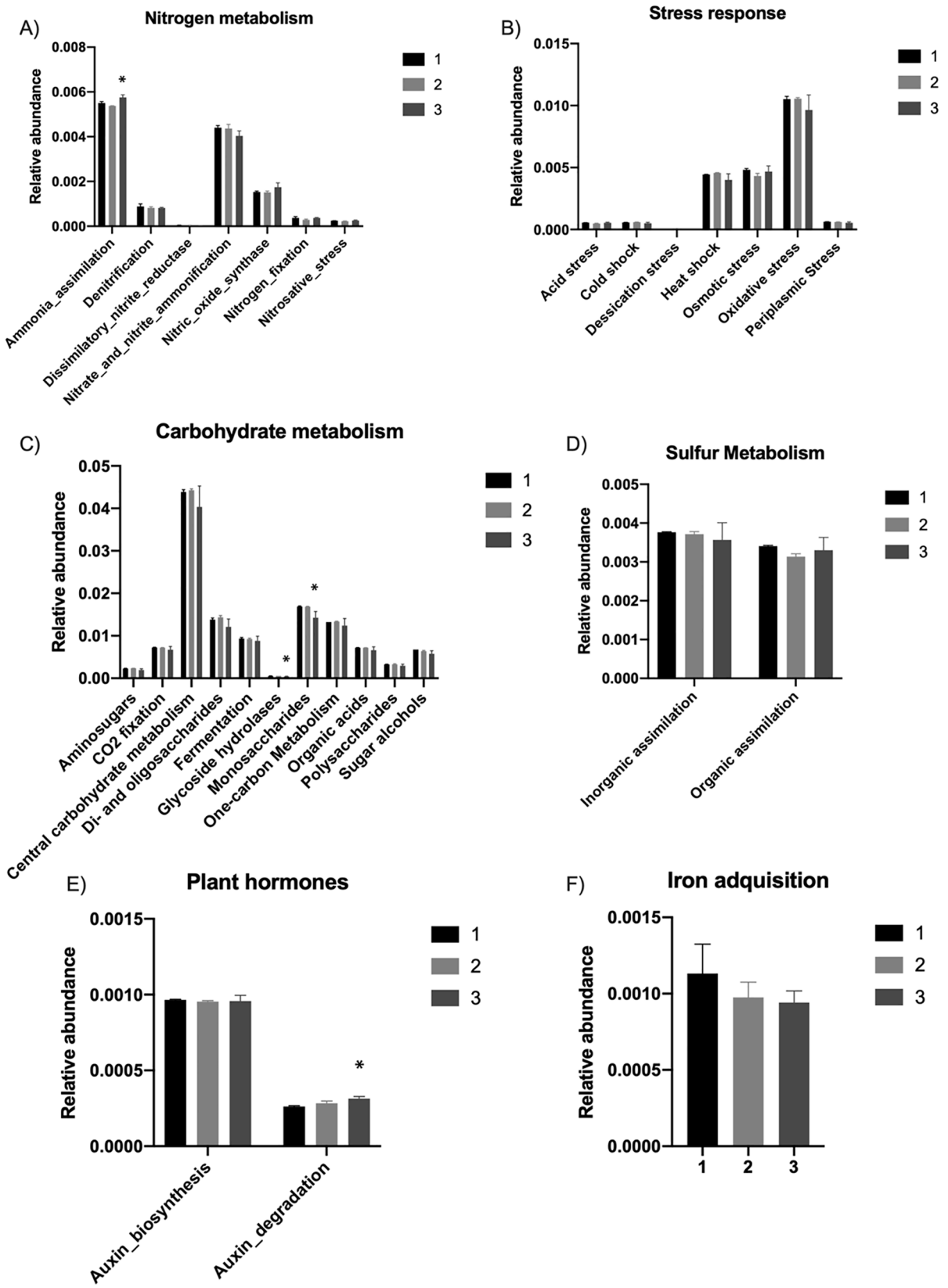

Fig. 9. Abundance of sequences affiliated to functional SEED Subsystems levels in the three experimental soil variants: (1) plow cultivation; (2) plow cultivation with manure; (3) no plow cultivation. A) Nitrogen metabolism (SEED Subsystems Level 3); B) Stress metabolism (SEED Subsystems Level 3); C) Carbohydrate metabolism (SEED Subsystems Level 2); D) Sulphur metabolism (SEED Subsystems Level 2); E) Plant hormones (SEED Subsystems Level 3); F) Iron acquisition (SEED Subsytems Level 2).

growth promoting bacteria *Solibacteraceae*, which protect plants against some fungal pathogens, e.g., *Fusarium oxysporum*, and participate in the biogeochemical carbon cycle in the soil (Zhang et al., 2017).

Barnard et al. (2013) have shown that the relative abundance of *Solibacteraceae* increases with increasing soil water content, probably the increase in the % share of this family may have been caused by the application of manure to the soil (No.2) which leaded to improvement water properties of soil compering to soils of Nos. 1 and 3.Soil No. 2 was also characterized by a high relative abundance of bacteria from the family *Comamonadaceae*, which is consistent with the results of Zhang et al. (2019). The *Comamonadaceae* are ubiquitous in the environment, and their abundance in arable soils increases under the influence of fertilization (Delgado-Baquerizo et al., 2018). Their presence in agricultural soils is very important because most of them promote the growth of plants (PGPB); they can increase plant tolerance to heavy metals and participate in the circulation of elements and in the decomposition of dead plant tissues (Belimov et al., 2005; Piotrowska-Seget et al., 2005; Lin et al., 2016; Chen et al., 2018, Ferreira et al., 2021).

A positive correlation was demonstrated between *Mycobacterium* and *Burkholderia*, which were present in the largest numbers only in the non-fertilized soil that had not been tilled for many years (No. 3). At the same time, these two groups of prokaryotes were negatively correlated with *Candidatus Solibacter* and *Gemmatimonas*, which were most abundant in the fertilized soil under plough tillage (No. 2). Bacteria of the genus *Gemmatimonas* are obligate aerobes; however, there are species among them, e.g., *G. aurantiaca*, which can reduce nitrous oxide (N_2O) in the soil environment (Doyoung et al., 2017). *Candidatus solibacter* synthesizes several enzymes involved in the breakdown of soil organic matter, releasing various nutrients into the soil. These bacteria also produce a biofilm from exopolysaccharides, which enables them to colonize mineral and organic particles in the soil. Thanks to the matrix of this substance, in which the threadlike cells of *Candidatus solibacter* are immersed, these bacteria can create channels through which they are able to take up nutrients from the soil. Additionally, under stressful environmental conditions, the biofilm is responsible for reducing the flow of moisture and nutrients in the soil (Pearce et al., 2012).

A strong positive relation was also found between *Sorangium* and *Bradyrhizobium*, with the highest values obtained for the soil not fertilized with manure but maintained under plow tillage for many years (No. 1). This may be due to the cooperation between these two groups of bacteria where *Sorangium* spp. hydrolysis various polysaccharides into simple sugars that are metabolized and used as a carbon and energy source by *Bradyrhizobium* sp. Additionally, *Sorangium* spp. carries out the dissimilatory reduction of nitrites to ammonium (Nelson et al., 2016), which can be assimilated by the free-living cells of *Bradyrhizobium* sp. inhabiting the soil environment (Table 4).

In the soil from variant No. 1, the increased numbers of bacteria from the group *Myxobacteria*, including *Sorangium* spp., and also of *Bradyrhizobium* spp. from the phylum *Proteobacteria* are very beneficial because these microorganisms promote plant growth (PGPB). The genus *Burkholderia* includes plant, animal, and human pathogens, as well as species that promote plant growth and development (PGPB), which bind molecular nitrogen, colonize the rhizosphere and phyllosphere of plants and the interior of their tissues (endophytes). Some produce fungicidal and fungistatic compounds and are therefore used as biocontrol agents (Eberl and, Vandamme, 2016). In the soil of variant No. 3, there was a noticeable positive correlation between *Burkholderia* spp. and *Mycobacterium* spp., which may be related to, *inter alia*, the rhizoexudates of the highbush blueberry plants growing in that soil. Metagenomic analyses have shown the occurrence of these groups of prokaryotes in the rhizosphere of blueberry plants (Li et al., 2020).

Fertilization with manure had increased in the soil the percentages of fungi of the orders *Sordariomycetes* and *Eurotiomycetes*, which mainly include plant pathogens, and which is thus unfavourable for the quality of the soil and the plants grown in it (Table 4, Fig. 4). This finding is in line with the research by Tayyab et al. (2019) and Wen et al. (2020). The results presented in this article are divergent from the study by Sun et al. (2016), who claim that the impact of manure on the diversity of fungi in the soil can vary and depends mainly on the origin and type of manure.

The metagenomic analyses showed the absence of mycorrhizal fungi, e.g., those of the phylum *Glomeromycota*, in the soils of all the experimental variants (Nos. 1, 2, 3). There was only a negligible percentage of fungal genera that include some species used for biocontrol, such as *Emericella* spp., *Neosartorya* spp., *Neurospora* spp., and *Filobasidiella* spp. (Table 5 and Fig. 4). Most of the fungal taxa at the rank of genus were quite strongly correlated with one another. Strong positive correlations were found in soil No. 2 between the abundance of pathogens of the genera *Talaromyces, Aspergillus, Pyrenophora, Penicillium, Nectria*, and fungi used in biocontrol (*Emericella* spp., *Neosartorya* spp.). The listed genera of fungi were by far the most numerous in the soil of variant 2. Variants 1 and 3 were characterized by much smaller numbers of almost all of the fungal genera listed, which may be related to the non-use of manure.

The main SEED subsystems level 1 comprised 28 major metabolic classes (Table 5), the most abundant annotated reads including to clustering-based subsystems (CBSS), carbohydrate metabolism, and amino acids and derivatives (Table. 5). Delmont et al. (2012) also showed the presence of several highly abundant CBSS in soil metagenomes. These are groups of functionally coupled genes (genes found proximal to each other in the genomes of diverse taxa) whose functional attributes are not well understood. The relatively high abundance of these subsystems across all the soil samples analysed, as well as other sequenced terrestrial soils, suggests that they could have significant function in soil ecosystems. Delmont et al. (2012) have suggested, considering above information the CBSS should be explored in future research efforts to understand the composition of each soil ecosystems.

The most interesting that the investigated plow tillage systems significant effects on the metabolisms functions of microorganisms in these soils including in nitrogen and carbohydrate metabolisms as well as plant hormones (Fig. 8). The greater activity of microorganisms that cause the assimilation of ammonia in the soil from under blueberry (No.3) compering to others tillage may be due to two reasons. First of all, in the soil that has not been cultivated for 40- years, a constant ecological balance, undisturbed by plow tillage, has developed, which may favour the multiplication of these microorganisms. In addition, there is an annual inflow of biomass from failing leaves and root degradation into the soil, which provides the supply of organic carbon as an energy source for microorganisms. Probably multiannual lack of plowing on the soil from under blueberry cultivation (No.3) lead to weaker oxygenation of soil, what result decrease of monosaccharides metabolism in this soil.

Microbiological communities in the investigated soils shows higher reaction on some stressful abiotic factors including redox potential, osmotic pressure and temperature of soil however differs are not statistically significant. The reaction of stress factors was the similar in each soil of three experimental variants. The no-tillage soil under blueberry cultivation shows lower reaction of oxidative stress compering to other (No. 1 and no. 2). This is due to the lower oxygenation of the soil environment due to the lack of mechanical cultivation (plowing).

Moreover, there were no significant differences in the level of biosynthesis of plant hormones, including auxins, in the three tested soils (No. 1, 2 and 3). On the other hand, statistically significantly higher auxin degradation was demonstrated in the no-plowing soil (No. 3) than in the other two examined soils (No. 1 and 2) (Fig. 1).

The sulphur metabolism in soil did not depend on the cultivation methods used and was similar in each of the experimental variants (No. 1, No. 2, and No. 3) (no significant differences were found). It is important from the point of view of the ecology of arable soils, as well as the cultivation of plants, as the three methods of soil cultivation used provide optimal sulphur uptake by plants, which is important for their growth and development. Additionally, it is very significant because

there is a deficiency of this element in world soils (Scherer, 2009).

The results our experiments show lack the affect plowing on the synthesis of auxins because there are no significant differences in the level of biosynthesis of auxins, in the three tested soils (No. 1, 2 and 3). Whereas statistically significantly higher auxins degradation was demonstrated in the no-plowing soil (No. 3) than in the plow soils (No. 1 and 2). This is an unfavourable process because the auxins synthetized by different taxonomic groups of microorganisms in soil environment have the significant ecological impact affecting plant growth and development (Kudoyarova et al., 2017).

Used three methods of tillage did not change the abundance of gene sequences related to iron acquisition in the analysed soil metagenomes, however their highest level (no statistical significance differences) occurred in the arable soil without the addition of manure (Fig. 9F). Iron is an essential element for most organisms, but it can be a life-limiting factor due to its insolubility under aerobic conditions at neutral pH. In response to this stress, some bacteria possess high affinity transport systems (Crosa, 1997) and produce high affinity siderophores that complex iron from the environment to optimize its recovery.

In summary, the study conducted has provided information on the microbial diversity of soils under specific soil conditions (luvisoils) in a temperate climate. This knowledge can be used not only to define whether a soil is “healthy” or “unhealthy, but also to estimate ecosystem services that take soil into account.

5. Conclusions

Soil microorganisms respond with changes in their abundance and taxonomic composition depending on the methods of soil cultivation.

Fertilization with manure (No.2), although it had improved the physicochemical properties of the soil and increased in the percentage of *Actinomycetes*, had nevertheless contributed to the increase in fungal pathogens.

Among the microorganisms in the soil fertilized with manure, *Actinobacteria* were the most abundant, while *Planctomycetes* were the least abundant; the uncultivated soil was dominated by *Nitrospirae* (No.3).

On the one hand, *Gemmatimonas* sp. can be recommended as microbiological indicators sensitive to lack of both plow cultivation of soil and organic fertilization, on the other hand *Mycobacterium* sp. is recommended as resistivity index on the long-term lack of plow cultivation as well as organic fertilization in moderate climate conditions.

Sorangium sp. could be recommended as microbiological indicators which responds by reducing the quantity under effect of the organically fertilized soil, while the plow and no-plow cultivation does not affect changes in its quantity in moderate climate conditions.

The use of various cultivation methods changed the biochemical functions of soil, including nitrogen and sulphur metabolism and carbohydrate metabolism, and in the production of plant hormones and siderophores. Additionally, soil tillage methods changed the response of microorganism’s stresses, including oxidative stress.

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CRediT authorship contribution statement

E.B. Górska: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **W. Stępien:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **A. Cunha:** Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing. **I.N. Sierra-Garcia:** Data curation, Formal analysis, Supervision, Validation, Writing – original draft, Writing – review & editing. **K. Szyszkowska:** Investigation, Validation. **D. Gozdowski:** Formal analysis, Methodology, Software, Validation, Visualization. **B. Gworek:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **L. Sas-Paszt:** Conceptualization, Resources, Supervision, Writing – review & editing. **A. Lisek:** Validation, Visualization. **E. Hewelke:** Supervision, Validation, Visualization. **A. Prędecka:** Supervision, Validation. **I. Olejniczak:** Supervision, Validation. **P. Trzciński:** Validation, Visualization. **A.H. Baczewska-Dąbrowska:** Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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