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Metagenomic characterization of soil microbial communities in typical chernozem of Moldova

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Abstract

Based on the new generation sequencing methods, such as pyrosequencing (Roche/454 Life Sciences), the metagenomic characteristics of typical chernozem microbiomes were determined and described for the first time in Moldova under different land use conditions. It was revealed that the community of prokaryotes is represented by both domains, particularly twelve bacterial phyla (61.4%) and one phylum *Archaea* (6.2%). Only nine phyla had an abundance above 1.0%: the archaeal phylum *Crenarchaeota* (*Thaumarchaeota)* and the bacterial phyla *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Acidobacteriota*, *Firmicutes*, *Verrumicrobiota*, *Platctomycecota*, and *Myxococcota*. Phyla, with a share of less than 1.0%, preferred the conditions of experimental variants of crop rotations. A significant proportion were unidentified microorganisms, the abundance of which varied from 25.8 to 38.2% in spring and from 27.9 to 40.2% in summer. The influence of soil and climatic conditions on the formation of the typical chernozem microbiome was more significant than agricultural practices.

Keywords: microbiome, soil DNA, sequencing, prokaryotes, typical chernozem.

1. Introduction

The soil metagenome is defined as the totality of microbial genomes found in the soil environment (Andronov *et al*., 2012; Angel *et al*., 2012; Daniel, 2005) or as the entire genetic material extracted directly from the soil (Gilbert & Dupont, 2011), which is considered a depository of soil diversity (Chernov et al., 2015; Zvyagintsev, 1987). The study of genetic material obtained directly from environmental samples (Andronov *et al*., 2012; Angel *et al*., 2012) is the main approach in soil metagenomics and a powerful tool in ecology for studying microbial communities (Rondon et al., 2000; Rosselló-Mora et al., 2001; Torsvik & Øvreås, 2002). Analysis of the total DNA of soil microbial communities makes it possible to theoretically determine the entire composition of the microbial population of biological samples, including non-cultivated microorganisms (Andronov et al., 2012). It does not have the shortcomings of methods for isolating cultivated microorganisms. Only by analyzing total DNA isolated from a soil sample can one judge the proper structure and composition of the microbial community, as well as its biological diversity (Ivanova *et al*., 2015; Kutovaya *et al*., 2015; Pershina *et al*., 2012).

The metagenomic approach has become possible due to modern technologies that allow the analysis of large amounts of genetic information through high-throughput sequencing (Bates *et al*., 2011; Caporaso *et al*., 2012; Costello, 2007). The most popular in metagenomic studies is the analysis of the 16S rRNA gene on the structure on which the modern phylogenetic classification of prokaryotic organisms is based (Aslam et al., 2012). Over the past years, many

modern metagenomic studies have been carried out as part of large international projects, including the Human Microbiome Project, which focuses on the study of human microbiomes, MEP (Microbial Earth Project), EMP (Earth Microbiome Project) and Terra Genome, dedicated to the study of soil metagenome, as well as others (Yilmaz *et al*., 2011; Vogel *et al*., 2009).

Analysis of the phylogenetic structure of microbiomes and identification of its relationship with the physical and chemical processes and features of soil genesis seems to be a promising way to solve the problems of the ecology of soil microorganisms concerning the relationship between the structure of microbial communities and soil properties (Gilbert & Dupont, 2011; Gray & Head, 2001; Hartmann *et al*., 2009; Howe *et al*., 2014). Identifying the features of the structure of prokaryotic communities associated with soil and climatic properties, combined with modern methods of high-throughput sequencing and the classical approach of genetic soil science, can be used as soil bioindicators (Ivanova *et al*., 2015; Pershina *et al*., 2012). Metagenomic studies in this area attempt to assess the relationship of the soil metagenome or its individual parts with the type of land use (Ivanova *et al*., 2015; Kutovaya *et al*., 2015; Zhelezova *et al*., 2015; Zvyagintsev, 1987), fertilization (Chernov *et al*., 2015), or a combination of different agricultural practices (Ivanova *et al*., 2015; Kutovaya *et al*., 2015; Pershina *et al*., 2012). Significant differences are found in the taxonomic structure of microbiomes in agricultural soils and soils under forest vegetation. Several researchers note a lower diversity of microorganisms in the soil of the forest zone (Chirak *et al*., 2013).

Agricultural use of land leads to long-term changes in the content of organic matter and nutrients, aggregate composition, pH value, and other indicators [8–10], which cannot but affect the structure of soil microbial communities. The soil metagenome, reacting to all these changes, can play the role of a biodiagnostic tool that makes it possible to assess the degree of influence of anthropogenic load and predict further changes in the ecosystem. Although the geography of works on soil metagenomics covers almost all regions of the Earth, there is no data on the metagenomic analysis of the soil cover in Moldova in the scientific literature, except for studies.

The aim of the research is to characterize the prokaryotic microbial community of a typical chernozem based on the analysis of their metagenome.

The task of the research included: 1 – identification of prokaryotes at the level of phyla, 2 – determination of the relationship between the phyla of the bacterial and archaeal domains, 3 – identification of prokaryotic phyla with the highest abundance, and 4 – determination of the developmental features of the dominant phyla on different backgrounds of typical chernozem.

2. Methodology

The object of research is the microbial communities of the typical chernozem of the Central soil-climatic zone of Moldova. The studies were carried out on two land use systems: in the forest belt and on the arable land of the "Biotron" long-term experimental station (Chisinau). The plots of the field experiment (arable lands) selected for the study were occupied by plants of two contrasting fodder crop rotations (with and without alfalfa) in three variants: 1 – unfertilized background (control); 2 – mineral background (mineral fertilizers); 3 – organic background (cattle manure) (Table 1). Fertilizers were applied depending on the crop in such a way as to compensate for the removal of NRK by plants, so the fertilizer doses in table 1 are not fixed. The amounts of organic fertilizers were calculated based on the proportion of 100 tons of manure instead of $N_{450}P_{109}K_{470}$.

The soil of the experimental plots is typical low-humus chernozem. The content of humus in the layer 0-60 cm is from 2.2% to 3.4%. The average initial content of P_2O_5 and K_2O in the 0–20 cm layer was 3.70 and 19.10 mg/100 g, respectively. The sum of absorbed bases in the 0-50 cm layer was 28-30 meq/100 g of soil, the reaction of the soil medium was weakly alkaline (pH = 7.8), the specific gravity of the soil was 2.6 g/cm 60%, and the bulk density was 1.06-1.30 g/cm^3.

The planning of the field experiment was carried out according to Dospekhov (1990) in 3 repetitions. The area of the experimental plots was 260 m^2. The standard in the comparative assessment of the microbiological state of the experimental variants was the soil of the uncultivated soil of the forest belt located next to the arable land. Soil samples 0-30 cm were taken in the spring and summer of 2020 according to microbiological requirements (Aseeva *et al*., 1991). Agrochemical analyses were carried out according to classical methods (Arinushkina, 1970). The metagenomic analysis of soil microbiomes was carried out in 2020 using high throughput sequencing technology, i.e. "reading" nucleotide sequences in DNA, using equipment of the Core Centrum 'Genomic Technologies, Proteomics and Cell Biology' in ARRIAM, St. Petersburg, Russia.

Table 1. Scheme of fodder crop rotation and fertilizer doses in long-term experiments on typical chernozem of the Station "Biotron"

Years of study	Experimental variants		
	Control	Mineral background	Organic background
Crop rotation with lucerne			
1995, 2002, 2009, 2016	Lucerne, year 1	According to the output NPK with crop: $N_{45\text{-}90}P_{30\text{-}60}K_{60\text{-}90}$	Manure 10-12 t/ha per year
1996, 2003, 2010, 2017	Lucerne, year 2		Aftereffect
1997, 2004, 2011, 2018	Lucerne, year 3		Aftereffect
1998, 2005, 2012, 2019	Winter wheat		Aftereffect
1999, 2006, 2013, 2020	Triticale/grains		Aftereffect
2000, 2007, 2014, 2021	Soybeans or peas Triticale (2021)		Aftereffect
2001, 2008, 2015, 2022	Winter wheat Maize/grains		Aftereffect
Crop rotation without lucerne			
1995, 2002, 2009, 2016	Fodder beet	According to the output NPK with crop: $N_{45\text{-}90}P_{30\text{-}60}K_{60\text{-}90}$	Manure 20-24 t/ha per year
1996, 2003, 2010, 2017	Soybeans or peas		Manure 10-12 t/ha per year
1997, 2004, 2011, 2018	Maize/silage		Aftereffect
1998, 2005, 2012, 2019	Winter wheat		Aftereffect
1999, 2006, 2013, 2020	Triticale/grains		Aftereffect
2000, 2007, 2014, 2021	Soybeans or peas Triticale (2021)		Aftereffect
2001, 2008, 2015, 2022	Winter wheat Maize/grains		Manure 10-12 t/ha per year

The year 2020 was characterized by insufficient rainfall. The spring drought lasted for the whole year. Soil moisture in the arable layer did not exceed 8-10%.

3. Results and Discussion

According to the analysis of nucleotide sequences at the domain level, most microbial communities (61.4%) comprise prokaryotes belonging to the Bacteria domain (Figure 1). Of these, about 1.9% were unclassified sequences and 1.0% were rare prokaryotes of the Bacteria domain (with an abundance of each less than 1.0%).

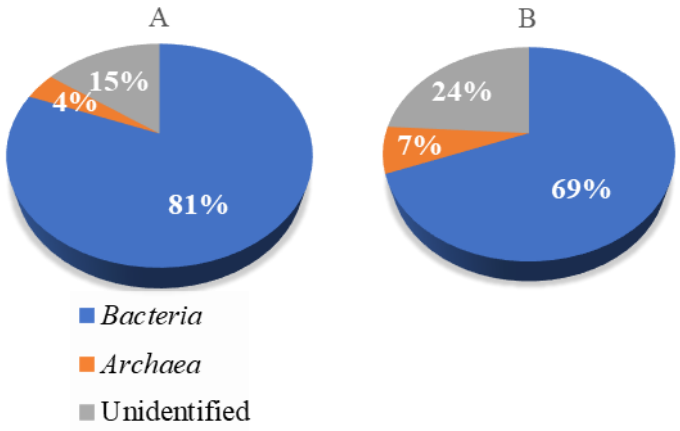

Figure 1. The proportion of bacteria and archaea detected in typical chernozem biomes during the season: A - spring, B – summer.

The abundance of archaea (6.2%) was inferior to the abundance of bacteria and was inversely proportional to the prokaryotes of the *Bacteria* domain. The correlation was more pronounced in spring (r = –0.4) than in summer (r = –0.3). The proportion of archaea compared to representatives of the *Bacteria* domain was about 10%. In addition to bacteria and archaea, all studied samples contained undetermined sequences, the proportion of which averaged 32.0%.

Analysis of nucleotide sequences at the phylum level revealed a similar taxonomic composition of prokaryotic communities consisting of 13 phyla (Figure 2). Domain *Bacteria* belonged to 12 phyla with the following average abundance: *Proteobacteria* - 21.2%, *Actinobacteriota* - 17.5%, *Acidobacteriota* - 4.3%, *Firmicutes* - 4.1%, *Bacteroidota* - 7.2%, *Fibrobacterota* - 0.1 %, *Verrumicrobiota* - 1.9%, *Planctomycecota* - 1.3%, *Gemmatimonadota* - 0.4%, *Myxococcota* - 1.3%, *Patescibacteria* - 0.2%, *Nitrospirota* - 0.4%, and the *Archaea* domain was represented by only one phylum *Crenarchaeota* (*Thaumarchaeota*) - 6.2%.

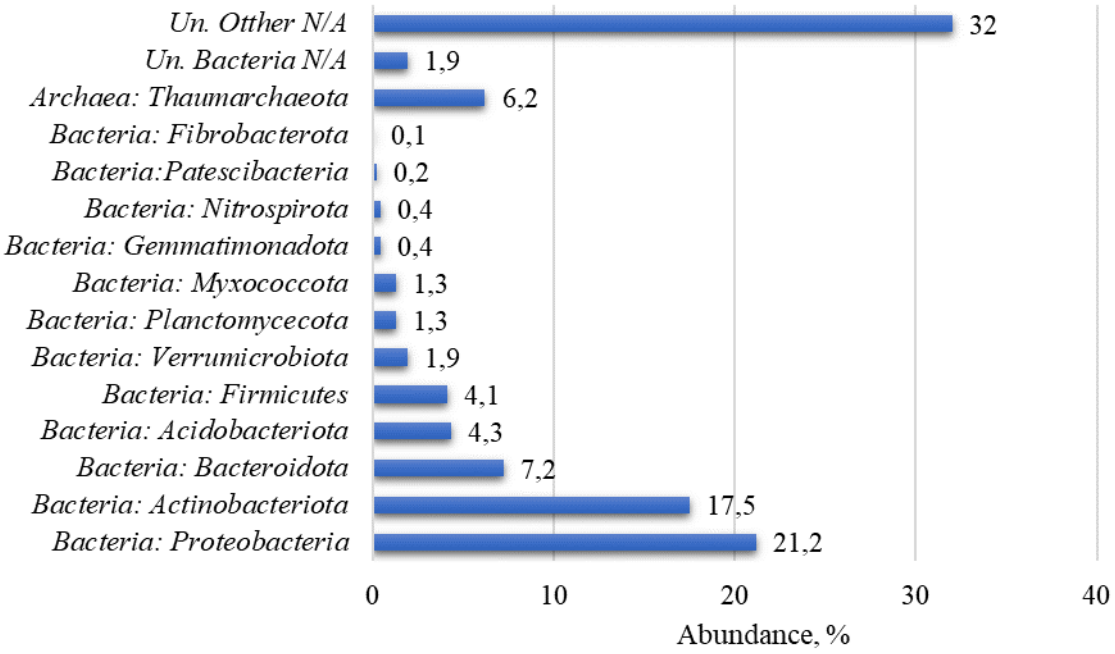

Figure 2. Taxonomic composition of soil prokaryotes at the level of phyla.

Comparative analysis at the phylum level showed some differences in the structure of the microbial community between the variants. The taxonomic composition of prokaryotes in the spring and summer periods differed both in crop rotations and on different backgrounds of typical chernozem. The largest number of phyla in spring was recorded on unfertilized and organic backgrounds of crop rotation without the participation of alfalfa - 13. The smallest number of phyla was also recorded in crop rotation without the involvement of alfalfa and in the uncultivated soil of the forest belt - 11. In summer, the maximum number of phyla was identified in all variants - 13, and only in the uncultivated soil of the forest belt - 11.

Thus, the prokaryotes of the phylum *Proteobacteria* recorded the highest indicators in spring, showing themselves approximately equally in the experimental variants (21.1-25.0%) and the soil of the forest belt (20.3%). The highest abundance was observed in the soil of the mineral background of both crop rotations. In summer, representatives of this phylum also met with the greatest abundance in the experimental variants of both crop rotations (18.9-24.0%). In the variant of the uncultivated soil of the forest belt, this abundance was 14.3% (Figure 3).

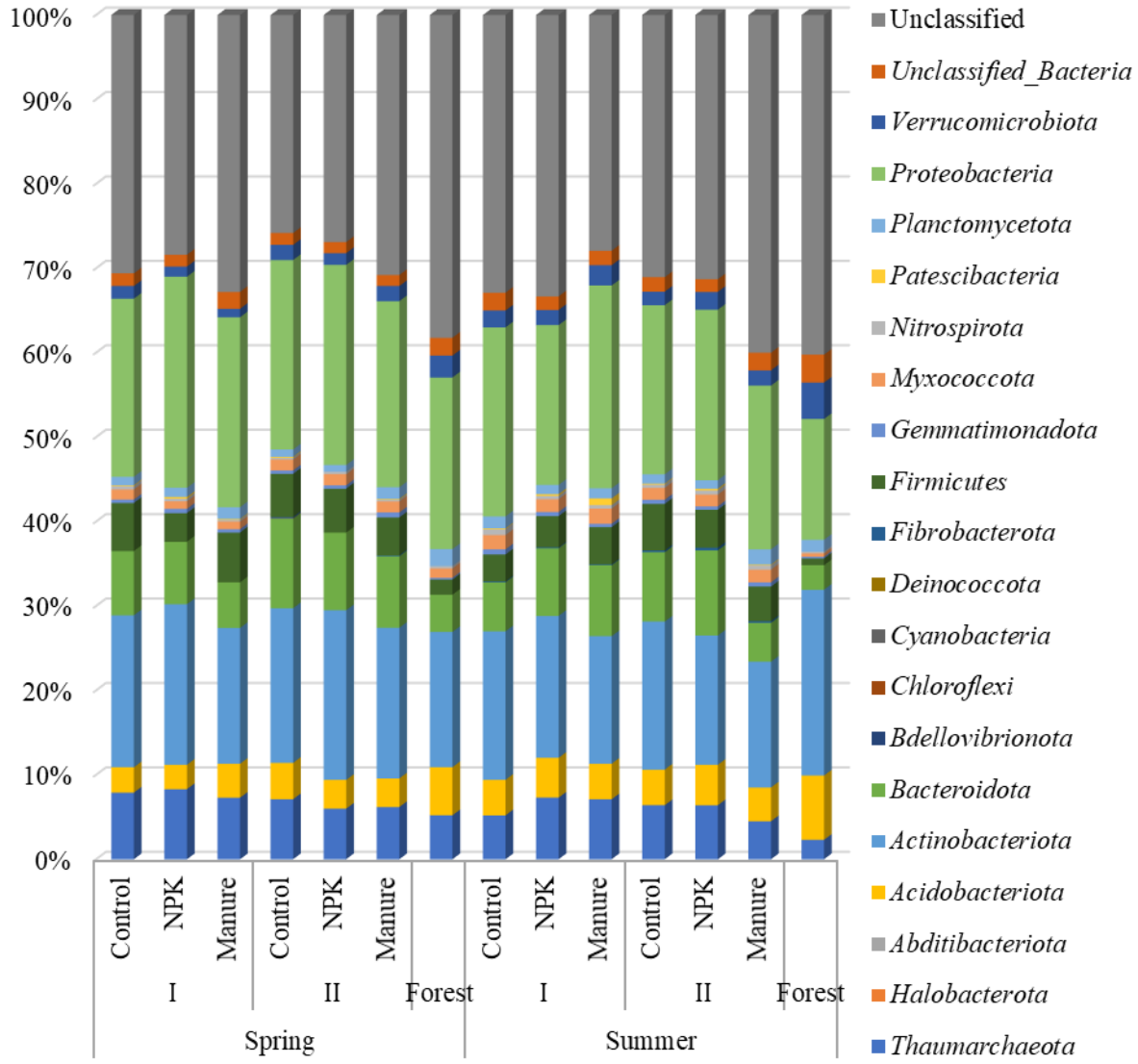

Figure 3. Taxonomic structure of microbiomes of typical chernozem (at the phylum level), I – crop rotation with alfalfa, II – crop rotation without alfalfa.

Representatives of the phylum *Actinobacteriota* in spring had the greatest abundance in the experimental variants of crop rotations, namely, in the soil of the mineral background of crop rotation without alfalfa; in the soil of the forest belt, their abundance was the lowest, 16%. In summer, their abundance was the highest in the soil of the forest belt - 22.0% and somewhat less in the soil of the experimental variants of crop rotation, 14.9-17.6%.

Bacteria belonging to the phylum *Bacteroidota* also preferred the conditions of experimental crop rotations in spring, namely, the mineral background of crop rotation without the participation of alfalfa. In the uncultivated soil of the forest belt, their abundance was the smallest, 4.4%. In the summer period, the prokaryotes of the phylum Bacteroidota preferred the conditions of unfertilized and mineral background crop rotation without alfalfa, 5.8-10.1%.

Less abundance was observed in the organic background of crop rotation without the participation of alfalfa, 4.6% and uncultivated soil of the forest belt, 2.9%.

Representatives of the phylum *Acidobacteriota* in spring preferred the conditions of the uncultivated soil of the forest belt (5.7%) and crop rotation without the participation of alfalfa, especially the conditions of unfertilized (3.0-4.3%) and mineral background (2.9-3.4%), then as in the organic background, a crop rotation soil with alfalfa was more preferred. In summer, on the contrary, they registered the highest indicators in the soil of the forest belt (7.6%) and the lowest in the soil of the experimental variants of crop rotations with a tendency to increase their abundance in the soil of the mineral background of both crop rotations (4.7-4.8%).

Microorganisms belonging to the phylum *Firmicutes* preferred the soil conditions of the experimental crop rotations in the spring, namely the soil of the unfertilized and organic background of the crop rotation with alfalfa (5.1-5.9%). Their smallest abundance was recorded in the uncultivated soil of the forest belt, 1.8%. In summer, these prokaryotes preferred the conditions of unfertilized and mineral background of crop rotation without alfalfa (4.0%-5.5%), with the lowest abundance in the soil of the forest belt, 0.8%.

Representatives of the phylum *Verrumicrobiota* preferred the soil conditions of the forest belt in spring, 2.6%. The soil of experimental variants of crop rotation was slightly low, 1.0-1.8%. The highest rates were observed in the soil of unfertilized and organic crop rotation background without alfalfa, 1.8%. In the summer period, they had the greatest abundance in the soil of the forest belt, 4.3%, and the least - in the soil of the experimental variants, 1.6-2.4%.

Representatives of the phylum *Planctomycecota* in the spring equally manifested themselves in the soil of the experimental variants of crop rotations (0.8-1.4%) with a slight tendency to increase in the crop rotation with alfalfa. The highest rates were observed in the soil of the forest belt, 2.0%. In summer, these prokaryotes were relatively similar in all variants, 1.1-1.4%, and only in the organic background of crop rotation without alfalfa, their abundance was 1.8%.

Microorganisms belonging to the phylum *Myxococcota* equally manifested themselves in the soil of the experimental crop rotations (0.9-1.3%) and the untreated soil of the forest belt (1.1%), with a tendency to increase in the soil of the crop rotation without the participation of alfalfa. In summer, the bacteria of this phylum were also relatively evenly represented in the experimental variants of crop rotations, 1.4-1.8%, with a tendency to increase the share of the crop rotation with alfalfa in the soil. In the uncultivated soil of the forest belt, their abundance was 0.4%.

Bacteria of the phylum *Gemmatimonadota* showed themselves better in the soil of experimental crop rotations in spring, 0.4-0.6%. In summer, they were relatively evenly represented in experimental variants (0.4-0.6%), with the lowest abundance in the uncultivated soil of the forest belt, 0.2%. In the non-cultivated soil of the forest belt, they were the least represented, 0.2%.

The phylum *Nitrospirota* was rare but manifested in the same way in the spring both in the soil of the experimental crop rotation (0.2-0.4%) and in the soil of the forest belt (0.3%). In summer, although representatives of this phylum were also rarely encountered, their abundance was equally represented in the experimental variants (0.4-0.7%) but less in the uncultivated soil of the forest belt.

Prokaryotes of the phylum *Patescibacteria*, also rare, preferred the soil of the experimental variants in spring (0.1-0.2%) and were not found in the soil of the mineral background of the crop rotation without alfalfa and in the soil of the forest belt. In summer, this

phylum had the greatest abundance in the organic background of crop rotation with alfalfa (0.2%) and was not found at all in the soil of the forest belt.

Bacteria of the phylum *Fibrobacterota* were rare; in spring, they showed themselves only in unfertilized and organic backgrounds of crop rotation without alfalfa (0.1%), while in summer, their abundance differed little in the variants, and they did not manifest themselves at all in the soil of the forest belt.

Archaea belonging to the phylum *Crenarchaeota* **(***Thaumarchaeota***)** have been characterized as prokaryotes with a dominant presence. In spring, they preferred the soil conditions of the alfalfa crop rotation, especially the variant with a mineral background. In experimental variants of crop rotation, their abundance increased (6.0-8.3%) compared to the soil of the forest belt (5.2%). In summer, they registered the highest values in experimental crop rotations (4.5-7.3%) and the highest in fertilized crop rotations with alfalfa (7.1-7.3%). In the soil of the forest belt, their abundance was the smallest, 2.3%.

Thus, using a high-throughput sequencing method with high resolution and the ability to study, including non-cultivated forms of microorganisms, for the first time, the metagenomic characteristics of the microbiomes of a typical chernozem of Moldova were determined and described, which is a reflection of the actual diversity of prokaryotic soil communities. The combination of the features of the chernozem soil and the plants are grown on it in fodder crop rotations led to significant changes in prokaryotic communities. According to the results of the analysis, these communities differ significantly from the communities of the original soil (Leininger *et al*., 2006; Paul, 2015; Pester *et al*., 2011).

The distinguishing features of the studied soil can be explained by the competition of microorganisms for the scarce resources of low-humus substrates of the studied chernozem, as well as their high adaptive potential to environmental conditions (Ivanova *et al*., 2015). In this case, we mean the traceable dependence of prokaryotes on the leading environmental factors, which, under the conditions under consideration, are abiotic indicators - insufficient humidity, high temperature, and poor nutrient content (Zvyagintsev, 1987).

The established individuality of the studied microbiomes made it possible to identify discrepancies in the taxonomic composition of the studied variants, possibly due to several reasons. First, this is due to the inhomogeneity of the chemical properties of the researched backgrounds (Zvyagintsev, 1987). Second, the reason for the heterogeneity of the microbiome is the heterogeneity of the soil as a habitat for soil microorganisms, as well as the localization of vegetative forms of microorganisms in microzones with available nutrients and more favourable conditions for maintaining their vital status (Chernov *et al*., 2015). The interaction between the living and non-living parts of the soil (Vernadskiy, 2004) determines the breadth of the ecological functions of the studied soil, causing the diversity of soil prokaryotes and thus forming differentiated communities of soil microorganisms in low-humus chernozems.

The results revealed that microbial community formation in chernozem ecosystems depends on physicochemical factors (Leininger *et al*., 2006; Paul, 2015; Pester *et al*., 2011). This is due to a lack of moisture and high temperature (drought), and a lack of nutrients in the arable layer of a typical low-humus chernozem.

4. Conclusions

Some conclusions emerge from this study as follows:

- Metagenomic analysis of prokaryotic communities showed the presence of 13 phyla belonging to the Archaean and bacterial domains. A significant proportion were unidentified microorganisms, the abundance of which varied from 25.8% to 38.2% in spring and from 27.9% to 40.2% in summer.
- Nine phyla were identified, a proportion above 1.0%: the archaeal phylum *Crenarchaeota* (*Thaumarchaeota*) and bacteria *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Acidobacteriota*, *Firmicutes*, *Verrumicrobiota*, *Plactomycecota* and *Myxococcota*. Phylums with a share of less than 1.0% preferred the conditions of experimental variants of crop rotations.
- The conducted studies showed that soil and climatic conditions on the formation of microbiomes of typical chernozem were more significant than agrotechnical measures.

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