Changes in prokaryote communities of southern chernozem under the influence of different farming systems

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Abstract. Soil microbiota or microflora is profoundly influenced by various physical, chemical, and biological environmental factors. Therefore, it serves as a good indicator of soil condition. With the introduction of new farming systems in agriculture, an intensive study and understanding of soil microbial community will make it possible to comprehensively analyze these systems including determining the degree of their impact on agroecosystems. The present study analyzed the structure of prokaryote communities in soil samples collected from the southern chernozem where different agricultural techniques are used, namely conventional, which involves plowing and a new method to the region, i.e., no-till farming. The outcomes were compared with the virgin land. The changes in soil metagenome were analyzed using high productive sequencing of 16S rRNA gene libraries. The authors found out that farming systems significantly influenced the changes in communities of prokaryotes of the southern chernozems, thereby creating conditions for the more active development of some groups of microorganisms while limiting others. A relative resistance to the effects of farming systems was noted for the genera Bacillus and Paenibacillus.

1. Introduction
Physiological interaction among microbes is considered a significant factor that affects soil fertility. Moreover, agricultural practices significantly affect the activity of microorganisms and conditions in which they exist, as well as cause changes in the ratio of ecologic and trophic groups. Agricultural systems based on conventional tillage are unable to provide optimal conditions to enhance soil fertility and moisture content in the root layer of soil, leading to irreparable damage to soil microbiota that intensifies erosion and soil degradation. Changes in the soil and climatic conditions in the Steppe make scientists search for farming systems with a more rational approach to natural resources [1]. From the ecological and economical perspectives, minimal tillage and no-till practices are being adopted in the Steppe zone of Crimea [2].
Nowadays, no-till is widely used in Latin America (Argentina, Brazil), Canada, and other countries. The no-till farming system leaves the stubble and crop residue coverage on the topsoil, which significantly contributes to energy saving, prevents moisture evaporation and reduces or eliminates soil erosion. This approach not only reduces soil disturbance through tillage but also preserves its structure and fertility, as well as decreases costs compared to conventional technology [3-6]. This agricultural practice was introduced in Crimea in 2006 and requires a careful study of its peculiarities under natural conditions of Crimean Steppe, which, according to experts, is the most promising area for no-till adoption.

A comprehensive study of soil microbial community will allow determining the degree of its impact on agro-ecosystems. The modern methods of molecular and genetic analysis have contributed to fully evaluate structural and functional features of soil microbiome [7,8]. The use of the next generation sequencing method (NGS) allows us to study the taxonomic structure of the soil microbiome of various ecological niches, as well as to predict the possibilities of farming systems’ optimization and biologization, no-till in particular [9, 10]. Findings gained in these studies will create a background not only for assessing soil conditions but also for choosing the best agro-technical strategy.

Thus, the present study analyzed the prokaryote communities in the soil samples of southern chernozem (Chernozems) where different agricultural techniques were used (conventional, which involves plowing, and new for our region—no-till farming) and the results were compared with the virgin land.

2. Materials and Methods

2.1. Soil and Climate Conditions

We studied the changes in the soil metagenome of southern chernozem soil (45°27'37.9"N, 33°20'24.7"E) that was subjected to conventional tillage (plowing) and no-till farming. The samples were collected from soil layer at a depth of 5 to 20 cm in early October before sowing the winter cereal crops. The typical weather conditions within the sampling period included average air temperature of 6.4 to 6.9 °C, precipitation of 63 of 72 mm, and ground frosts up to −1 °C. In areas employing the no-till farming system since 2006, plots where plowed to a depth of 22 to 24 cm. In addition, the virgin land was chosen for comparison in the research. Legumes were cultivated in all land areas. The studied plots of southern chernozem were located in the arid Steppe zone of the Crimea, where the frequency of months with drought (hydrothermal coefficient ≤ 0.5) is 60 to 70% [11]. The samples at the time of DNA extraction had no significant differences in moisture content. The southern chernozem was weakly alkaline (pH: 7.5–8.0).

The stationary experiment on studying the conventional farming system (CFS) and no-till had been conducted since 2015 on the fields of FSBSI “Research Institute of Agriculture of Crimea” (45°31'47.3"N 34°11'48.0"E) under conditions of the Crimean Steppe. Sampling for analysis was carried out in 2018. The soil of the virgin land was used as a control.

Soil - southern chernozem low-humic on loess-like light clays. The climate of the steppe zone is arid, moderately hot, with mild winters, the average annual air temperature is 9.7–10.5°C, the hydrothermal coefficient is 0.7.

2.2. DNA Extraction

DNA was isolated from 0.5 g of frozen soil after mechanical destruction using zirconium beads in an extraction buffer. The extraction buffer consisted of following components: 350 μL of solution A (sodium phosphate buffer: 200 mM; isothiocyanate guanidine: 240 mM; pH: 7.0), 350 μL of solution B (Tris-HCl: 500 mM, SDS: 1% w/v, pH: 7.0), and 400 μL of phenol-chloroform mixture (1:1). The sample was disrupted for 1 min at the maximum regime of the equipment FastPrep-24 (MP Biomedicals, Santa Ana, CA, United States). The obtained samples were centrifuged at the maximum speed for 5 min. The liquid phase was collected and re-extracted with chloroform. DNA was precipitated by adding an
equal amount of isopropanol. After centrifugation, the precipitate was washed with 70% ethanol and dissolved in water at 65 °C for 5 to 10 min. DNA was purified by electrophoresis in 1% agarose gel. Further, DNA was isolated from the gel by sorption method on silicon monoxide [12].

2.3. Sequencing

For constructing and sequencing amplicon libraries, the purified DNA (10–15 ng) was used as a template in a PCR reaction. The PCR reaction consisted of 30 cycles with following steps: 95°C for 30 s; 50°C for 30 s; and 72°C for 30 s. The enzyme used was Encyclo polymerase (Eurogen, Moscow, Russia) with universal primers to the variable sequence of V4 gene 16S rRNA: F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGACTACVSGGGTATCTAAT) [13]. The libraries were prepared and sequenced on a MySeq (Illumina, USA) according to the manufacturer's instructions.

2.4. Taxonomic Analysis

taxonomic analysis of nucleotide sequences of amplicon libraries was conducted using Bioconda [14], QIIME [15] and PastPaleo [16] software. The analysis consisted of library separation by identifiers, verification of sequencing quality and filtration of nucleotide sequences, combining sequences into operational taxonomic units (OTU) using 97% similarity threshold. OTU taxonomic identification was performed using the RDP databank (http://rdp.cme.msu.edu/) [7].

3. Results

application of both agricultural techniques, namely plowing and no-till, increased the proportion of archaea (phylum Crenarchaeota) up to 17.9 to 18.6%, whereas the percentage was 10.8% in the virgin southern chernozem (Figure 1).

Figure 1. Percentage of dominant phil of prokaryotic communities in the soil samples of southern chernozem soil under different farming systems, according to data of metagenome analysis of 16S rRNA.

The current research established that bacteria prevailed in the southern chernozem, where the phyla, Proteobacteria and Actinobacteria, were dominant. Among the aforementioned bacteria, the representatives of the phylum Proteobacteria, with a share of 43.5% in the virgin soil, were predominantly present. Plowing contributed to its increase up to 46.3%, whereas no-till led to its decrease up to 36.4%.

No-till contributed to an increase in the proportion of Actinobacteria to 34.9% that could be a result of the accumulation of plant residues. Plowing accounted for 27.4% of Actinobacteria, lower than their percentage in the virgin land (30.6%).

The share of representatives of other phyla such as Acidobacteria, Chloroflexi, Verrucomicrobia, Firmicutes, Planctomycetes, Gemmatimonadetes, Bacteroidetes and Nitrospirae declined under the
influence of no-till and plowing up to 10.8% and 8.0%, respectively, whereas the share was 15.1% in the virgin land. A higher proportion (2.7%) of phylum Acidobacteria was noted in the virgin soil of southern chernozem, whereas it declined to 1.7% and 1.3%, respectively, under the influence of no-till and conventional tillage. The percentage of representatives of phylum Chloroflexi in the virgin soil of southern chernozem reached 3.0%. The plots with different farming systems (plowing and No-till) contributed to 1.3% abundance of this phylum by providing close to natural conditions for their development. The bacteria belonging to the phylum Verrucomicrobia represented 2.4% of the total microbial population in the virgin soil of southern chernozem. The plots with plowing and no-till induced to change natural conditions for the development of representatives of this phylum, their percentage decreased significantly and amounted to 0.2%. Similar decreases in the representation of prokaryotes in agrocenoses in comparison with virgin soil were observed among phyla Chloroflexi, Planctomycetes, and Gemmatimonadetes. The share of phyla Chloroflexi and Gemmatimonadetes in virgin lands was 3.0% and 2.8%, respectively. It was 1.7% and 2.6%, respectively, when the no-till system was employed, whereas conventional tillage (plowing) accounted for 1.3% and 1.9%, respectively. No-till farming system contributed to an increase in the proportion of phylum Bacteroidetes to 1.3% and 0.8% in the virgin land, whereas plowing led to a decrease of up to 0.5%.

The unknown sequences at different taxonomic levels were observed in the soil samples because of the incomplete database. At the level of the phylum, their share was 1%, whereas, at the family level, unidentified microorganisms accounted for 19%. The analysis of data from advanced sequencing of 16S rRNA gene libraries of soil samples revealed the diversity of representatives of more than 60 families (Figure 2). Both archaea—Nitrososphaeraceae (10.8–18.3%) and bacteria—Comamonadaceae (16.4–19.3%) and Alcaligenaceae (9.5–20.6%) were reported abundantly. Among taxonomic groups of microorganisms of the southern chernozems, families belonging to the phylum Proteobacteria were the most abundant. Those belonging to the Comamonadaceae accounted for 16.3% using the no-till technique, whereas plowing and virgin soil reported their level to be 19.3%.

![Figure 2. Diversity of the prokaryotic families based on 16S rRNA gene sequencing in the southern chernozem under different farming systems.](image-url)

No-till technique decreased the share of the Alcaligenaceae family to 9.5% in comparison to the virgin soil (15.7%). The representatives of this taxonomic group were abundantly present in the soil that was plowed, up to 20.6%. The two agriculture practices decreased the share of the family Hyphomicrobiaceae to 0.3% in comparison with the virgin soil (1.4%). The share of the family Spingomonadaceae was maximum (1.1%) in the soil treated with the no-till system and minimum (0.5%) after plowing. The percentage of representatives of the family Syntrophobacteraceae was 0.4% in all soil samples.
The families Bradyrhizobiaceae and Rhizobiaceae, include nitrogen-fixing bacteria and play a pivotal role in the productivity of agroecosystems. The average percentage of the representatives of family Bradyrhizobiaceae in the virgin soil was 0.8%. The maximum proportion of microorganisms of this family (1.4%) was observed when no-till technology was used and minimum (0.6%) after plowing.

The share of representatives of Cystobacteraceae and Halangiaceae are known as mucous soil bacteria, and Enterobacteriaceae family (included some plant pathogens and agronomically useful species) in the samples of southern chernozem did not exceed 0.1%.

Metagenomic analysis of microbial communities at the genus level showed the dominance of representatives of Rubrobacter and Achromobacter (table 1). Genus Rubrobacter belongs to the family Rubrobacteraceae and phylum Actinobacteria. It accounted for 5.5% in the virgin soil and its share increased under the influence of farming systems up to 10.5% on plowed land and up to 12.0% using the no-till technology. More representatives of the genus Achromobacter, which belongs to the family Alcaligenaceae and phylum Proteobacteria, were present in the virgin soil (15.4%). Plowing contributed to an increase in their share to 20.1%, whereas no-till led to a decrease of up to 9.1%.

Share of Bradyrhizobium in virgin soil was 0.7%, whereas it decreased to 0.1% under both systems of agriculture. Cultivation techniques also reduced the representation of the genera Candidatus Solibacter, Pseudonocardia, Kribbella, and Methylibium. The representatives of the genera Candidatus Koribacter, and Kribbella were noted only in the virgin soil (0.2%).

### Table 1. Percentage of different taxonomic groups of microorganisms (genus) in southern chernozem, according to the metagenome analysis of 16S rRNA

| Taxonomic group, genus | Percentage, % | Taxonomic group, genus | Percentage, % |
|------------------------|---------------|------------------------|---------------|
|                        | No-till       | conventional tillage   | virgin soil   |
|                        |               |                        |               |
| Candidatus             | 17.9          | 18.3                   | 10.8          |
| Nitrososphaera         | 0.3           | 0.2                    | 0.6           |
| Candidatus             | 0.0           | 0.0                    | 0.2           |
| Solibacter             | 0.4           | 0.3                    | 0.0           |
| Candidatus             | 0.3           | 0.0                    | 0.0           |
| Koribacter             | 0.2           | 0.0                    | 0.0           |
| Agromyces              | 0.2           | 0.2                    | 0.0           |
| Arthrobacter           | 0.0           | 0.0                    | 0.2           |
| Kiribella              | 0.1           | 0.0                    | 0.0           |
| Nocardoides            | 0.2           | 0.2                    | 0.3           |
| Pseudonocardia         | 0.2           | 0.1                    | 0.1           |
| Streptomyces           | 12.0          | 10.5                   | 5.5           |
| Flavisolibacter        | 0.3           | 0.0                    | 0.1           |
| Paenibacillus          | 0.1           | 0.0                    | 0.1           |
| Bacillus               | 0.4           | 0.3                    | 0.2           |
| Nitrospira             | 0.1           | 0.1                    | 0.0           |
| Balneimonas            | 1.0           | 0.3                    | 0.0           |
| Bradyrhizobium         | 0.1           | 0.1                    | 0.7           |
| Roseomonas             | 0.2           | 0.1                    | 0.0           |
| Skermanella            | 0.5           | 0.1                    | 0.0           |
| Kaistobacter           | 0.4           | 0.2                    | 0.5           |
| Sphingomonas           | 0.2           | 0.1                    | 0.2           |
| Achromobacter          | 9.1           | 20.1                   | 15.4          |
| Methylibium            | 0.1           | 0.0                    | 0.2           |
| Janthinobacterium      | 0.3           | 0.2                    | 0.1           |
| Ralstonia              | 0.2           | 0.1                    | 0.1           |
| Pseudomonas            | 0.6           | 0.2                    | 0.1           |
| Steroidobacter         | 0.1           | 0.1                    | 0.0           |
| Lysobacter             | 0.1           | 0.1                    | 0.0           |

Our analysis showed an increase in the proportion of representatives of the genera Bacillus, Janthinobacterium, and Pseudomonas under the influence of both systems of agriculture. The presence of plant residues on topsoil positively affects the genus Pseudomonas, as well as others [17]. The maximum value was obtained with the application of no-till practice. For instance, the maximum proportion of the representatives of genera Geodermatophilus, Streptomyces, Flavisolibacter, Ralstonia, Arthrobacter, and Phycicoccus was observed after no-till.
Thus, the present study assessed the changes in the soil microbiome using high productive methods of sequencing of 16S rRNA gene libraries on plots of southern chernozem (Chernozems) where different agricultural techniques were used (conventional, which involves plowing, and new for our region—no-till) and were compared with virgin lands.

Overall, the study included representatives of 14 phyla, 62 families, and more than 100 genera in the southern chernozem. The representatives of phyla **Proteobacteria** (36.4–46.3%), **Actinobacteria** (27.4–34.9%), and **Crenarchaeota** (Archaea, 10.8–18.3%) predominated the soil samples.

A total of 745 OTU were detected at the genus level according to the results of the NGS libraries of the 16S rRNA gene in southern chernozem in 2018. **Candidatus Nitrososphaera** prevailed in the identified taxa; its share in virgin soil reached 7.3%. With conventional tillage its level increased by 1.9% while using direct seeding it decreased by 1.1%. Representation of the genera **Segetibacter**, **Skermanella** and **Rubrobacter** under the influence of farming systems varied in proportion to **Candidatus Nitrososphaera**. The share of representatives of the genus **Flavisolibacter** using CFS and no-till increased by 0.3 and 0.7% compared to virgin land (1.3%). Representatives of **Balneimonas** reached 1.84 in virgin soil and 1.87% in the soil after conventional form of tillage, the decline was noted after no-till by 0.53 and 0.56%, respectively. The share of **Bacillus** almost did not change using no-till technology (1.8%), while it increased by 1% using CFS.

The index of Shannon alpha diversity decreased by 0.20 (conventional farming system) compared to no-till (4.77) and slightly differed from the microbial diversity in the virgin soil – 4.61. The same differences were observed after evaluating beta-diversity: the Whittaker index was 0.096 for CFS, 0.141 for no-till, and 0.119 for virgin soil. Simpson index and Evenness differences in the samples studied are not revealed (table 2). Also, the separate group was metagenome of virgin soil prokaryotes.

**Table 2.** Beta- and alpha- diversity of southern chernozem at the genus level under conditions of various farming systems and virgin soil based on the analysis of the main coordinates with the Bray-Curtis metric. NB: no-till - direct sowing technology, CFS - traditional farming system, VS - virgin soil.

| Index      | no-till | CFS       | VS         |
|------------|---------|-----------|------------|
| Simpson    | 0.98±0.00 | 0.98±0.00 | 0.98±0.00 |
| Shannon    | 4.77±0.06 | 4.58±0.04 | 4.61±0.04 |
| Evenness   | 0.22±0.01 | 0.19±0.01 | 0.19±0.01 |
| Whittaker  | 0.141   | 0.096417  | 0.11868    |

4. **Discussion**

The current study demonstrated through 16S rRNA gene sequence-based metagenome analysis that farming systems significantly influenced the changes in the communities of prokaryotes of the southern chernozem. An increase in the percentage of archaea to the total number of microorganisms could be attributed to the limiting factors for bacteria. **Archaea** are known to have a higher potential for adaptation under stress conditions, that is reflected in the narrowing of the ratio of these two domains [18].

The most common in abundance of representatives of soil bacteria phyla are **Proteobacteria**, **Actinobacteria**, **Acidobacteria**, **Verrucomicrobia**, **Chloroflexi**, **Planctomycetes**, **Gemmatimonadetes** and **Firmicutes** [19]. As shown Mummey et al. [20] and Pershina et al. [21] in technology-contaminated environment the percentage of **Proteobacteria** is reached up to 70%. In our research, the proportion of this phylum in 10-years No-till site less than in perennial Conventional tillage site and evidenced of a more stable microbiocenosis. **Actinobacteria** have the ability to decompose complex organic matter with increased resistance to a low moisture content in the environment [22]. Overall, the two agriculture practices contributed to an increase in the share of archaea and a decline in the representatives of other phyla. The representatives of phylum **Acidobacteria** are more sensitive to the soil pH than to other environmental parameters, including temperature, soil moisture, and nutrients [23].
Our results also showed that in the southern chernozem the dominant position was occupied by the major groups (the proportion above 10%) of bacterial communities of phyla Proteobacteria, Actinobacteria and phylum Crenarchaeota (Figure 1). The proportion of representatives of phyla Gemmatimonadetes, Chloroflexi and Acidobacteria was above one. The remaining fillets were among the minor groups of bacteria, the proportion of which did not exceed 1%.

The bacteria belonging to Cystobacteraceae and Halangiaceae families that grow on insoluble organic substrates. Some of them are producers of important medical antibiotics. The representatives of Enterobacteriaceae family, such as Klebsiella and Shigella, include some plant pathogens. There are also some agronomically useful species in this family. For example, Enterobacter nimpressuralis produces plants hormones, organic acids, and alkaline phosphatase; it positively influences plant growth and development and is utilized as a bio-fertilizer in agricultural production [24].

Earlier studies reveal that the bacteria of Achromobacter have the ability to decompose some herbicides, mostly those containing glyphosates [25], as well as other organic compounds [26]. Therefore, these are widely used as microbial fertilizers for soil remediation [27].

The structure of a bacterial community depends on chemical properties of the soil [28]. The representatives of genera Bacillus, Pseudomonas, and Rubrobacter are resistant to impact by agronomic techniques (cultivated crop, crop rotation, and liming) under sod-podzolic soil conditions, whereas Rhizobium and Arthrobacter are sensitive to these factors [29]. The studies of southern chernozem have shown that genus Bradyrhizobium is sensitive to agricultural practice.

We established that the farming systems significantly influenced the changes in the soil microbiome. Both farming practices, viz., conventional tillage and no-till farming, contributed significantly to an increase in the share of Archaea and decrease of bacterial phyla Chloroflexi, Acidobacteria, and Verrucomicrobia, among others. No-till farming system increased the presence of bacterial phylum Actinobacteria, which could be attributed to the accumulation of plant residues. Tillage in the southern chernozem reduced the proportion of bacteria of phylum Actinobacteria and increased the number of representatives of phylum Proteobacteria.

As a result of the taxonomic structure of soil microbiome study, 745 OTU were determined at the genus level. Maximum biodiversity was established for no-till. Seven dominant genera were identified: Candidatus Nitrosoaphaera, Rubrobacter, Flavisolibacter, Segetibacter, Bacillus, Balneimonas, Skermanella. The proportion of Candidatus Nitrosoaphaera was the highest among all representatives of the genera. Under the traditional farming system, it increased by 1.9% compared to virgin soil (7.3%). Consequently, the use of various farming systems led to a change in the structure of the southern chernozem microbiome.

Thus, the current study revealed that conventional tillage, which involves plowing, and no-till created favorable conditions for the active development of some taxonomic groups of microorganisms while limited others in the southern chernozem.

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