The taxonomic structure of southern chernozem at the genus level influenced by microbial preparations and farming systems

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Abstract. The taxonomic structure of the microbiome of southern chernozem compared to virgin soil was studied in the stationary experiment on the use of microbial preparations in the conditions of traditional for the steppe part of the Crimea and each culture of the five-field crop rotation of the farming system and no-till. Among 823 genera it was revealed 7 dominant ones: Candidatus Nitrososphaera, Rubrobacter, Flavisolibacter, Segetibacter, Bacillus, Balneimonas, Skermanella. The low level of cellulose-decomposing bacteria of the genus Cellulomonas was remarked in southern chernozem. The highest proportion of the representatives of this genus was observed in virgin soil (0.55 %), while the farming systems contributed to its decline in 1.3-1.6 times. Microorganisms of the genera Erwinia and Xanthomonas with phytopathogenic properties were found with a minimum proportion in communities from 0.003 % to 0.014 % and 0 – 0.005%, respectively.

The complex metagenomic analysis of the taxonomic structure of the soil microbiome allowed us to establish that the use of microbial preparations reduces the negative impact of the no-till farming system on the microbiocenosis of southern chernozem and improves its environmental feasibility in the Crimean steppe.

1. Introduction
Soil microorganisms play an important role in a variety of processes aimed at the formation and maintenance of soil fertility, have the ability to provide the plant with nutrients, protection from adverse factors, including phytopathogens, and stimulate its growth and development [1, 2]. They respond to changes in living conditions, which allows them to be used as indicators of the state of the environment. One of the factors causing changes in the composition of microbial communities of agroecosystems is the farming systems [3]. In the steppe conditions, where insufficient rainfall and high temperatures during the growing season of field crops reduce their productivity, the interest in the no-till farming system increases, due to its economic and environmental feasibility [4].

Currently, the use of the method of high-performance sequencing of the 16s rRNA gene allows expanding knowledge about the taxonomic structure of microbiomes of different ecological niches, to show their changes under the influence of anthropogenic factors.
When forming the rhizosphere microbiome in agrocenoses, the basis is soil microorganisms, whereas the introduced strains by seeds inoculation can change its composition [5]. Today there are promising studies in the management strategy of plant microbiomes aimed at improving the productivity and stability of agrocenoses, the quality of the products [6], as well as at solving the problems of biologization of plant growing technologies in different farming systems [7-9], in particular at no-till [10]. The Department of Agricultural Microbiology of the Crimean Research Institute has developed complexes of microbial drugs for crops cultivated in the region, used by presowing inoculation of seeds.

Our research aimed to study the effect of microorganisms-bioagents of microbial preparations on the taxonomic structure of prokaryotes at the level of the microbiome genus of southern chernozem under the conditions of different farming systems (traditional for the region and no-till).

2. Materials and methods

The stationary experience on the comparative analysis of microbial drugs influence in the conditions of traditional for steppe part of the Crimea and each culture of five-field crop rotation system of agriculture and no-till was firstly applied in 2015 on the fields of FSBSI "Research Institute of Agriculture of the Crimea" (the village of Klepinino of Krasnogvardeysky district, 45°31’47.3” N 34°11’48.0” E) (Figure 1).

Crop rotation under the traditional farming system (TS) included pure steam, winter wheat, oilseed flax, winter barley, grain sorghum; but at no-till, there is one difference – in the first field, peas are sown. The complex of microbial preparations (CMP) developed for each crop rotation culture was used by inoculation of seeds. The drugs which used as CMP for sorghum were the following: Biopolycid (the main function is to protect plants from phytopathogens), Phosphoenterin (growth-promoting and phosphate mobilization), Diazophyte (fixation of molecular nitrogen) and arbuscular mycorrhizal fungi (AMF) for improving the mineral nutrition (phosphorus, potassium, nitrogen) of plants. The inoculation with CMP was carried out at the rate of 2% of the seed weight, AMF was introduced at sowing - 100 kg/ha. The samples were selected in June, 2018. The type of soil is low-humous southern chernozem on the loess light clays. The thickness of the humus horizon is up to 40 cm, the entire humus layer - up to 70 cm. The amount of humus (Tyurin's method) - 2,00-2,20 %, movable phosphorus (Machigin's method) – 4,00–4,20, potassium exchange – up to 40 mg per 100 g of soil. Granulometric composition of southern chernozems is silty large-clay The number of water-
proofing aggregates that are larger than 0.25 mm in the humus horizon of virgin soils is 72-77 %. The content of agronomically valuable aggregates that are larger than 1 mm is 33-42 %.

The density of addition (bulk weight) in the plowing layer is 1,14–1,28, and in the subsurface one – 1,33–1,48 g/cm$^3$. The change in the total pore space is inversely proportional to the change in the bulk density. The total pore space of the upper horizons is 50,2 %, that is a satisfactory indicator, according to the agronomic assessment. The water-retaining property of the soils is high enough, they can accumulate more than 300-350 mm of moisture, but the reserves of productive moisture available to plants are only 160-180 mm.

As a reference, samples of the virgin steppe area were selected, the proportion of cereal components on which is about 80 %. On the surface of the soil, a thick layer of litter developed, the thickness of which reaches an average of 15 cm.

The climate of the steppe zone is arid, moderately hot, with moderately mild winters, hydrothermal coefficient (HTC) is 0.7. The average annual temperature is 9.7-10.5 °C. In July, at noon, the temperature rises to 28.9-30.2 °C, and in some years to 40-42 °C. The average minimum annual temperature ranges from -19 to -23 °C. In winter, the temperature may drop to -31 °C. The growing thaw is possible in 35% of winters. The frost-free period is 171 days. The sum of temperatures above 10 °C reaches 345 mm, of which 285 mm falls during the active vegetation period. The annual evaporation is 843 mm. East (22 %) and North-East (20 %) winds prevail. Strong winds are 28-30 days a year. The number of days with hot dry winds is from 10 to 19. The probability of drought for most of the territory is 40-50 % years [11].

High-productive sequencing of 16s rRNA gene libraries was used to study the taxonomic structure of the soil microbiome. DNA from soil samples was isolated using PowerSoil DNA IsolationKit (MoBio, the USA) according to the Manufacturer's Protocol Vortex Genee-2 (Mo-Bio, USA) was used for homogenization of samples. DNA purification was carried out electrophoretically followed by extraction from agarose gel [12]. The purified DNA was used as a matrix in the PCR reaction when creating amplicon libraries. The universal primers were also applied to the variable site V4 of the 16s rRNA gene – F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGACTACVGGGTATCCTAAT) [13]. The genes were sequenced using MySeq (Illumina, the USA) based on the resource center "Genetic technologies" of St. Petersburg University. The taxonomic and statistical analyses of the results obtained using the Bioconda software packages [14], QIIME [15], PAST Paleo [16] and the RibosomalDatabaseProject (RDP) database were used.

3. Results and discussion

Among 823 OTU genera in southern chernozem it was revealed 7 dominant ones: Candidatus Nitrososphaera, Rubrobacter, Flavisolibacter, Segitibacter, Bacillus, Balneimonas, Skermanella (tabl. 1). For Candidatus Nitrososphaera, econishes with a wide range of pH and temperature are available. Their proportion in the southern chernozem cultivated according to the traditional farming system was critical among the studied samples and amounted to 9.197%, which is 1.3 times more than in virgin soil and 1.5 times when using no-till. The use of microbial preparations contributed to its increase in both systems of agriculture.

Among the dominant genera of southern chernozem proved to be Rubrobacter. This genus of Actinobacteria has a high radiotolerance and it is common in desert soils [17]. The proportion of its presence in southern chernozem of the virgin area was 3.10%. Its insignificant increase (0.4%) was noted in the conditions of the traditional farming system and its inverse situation in the conditions of the no-till farming system. Probably, this tendency is due to the fact that the soil gets warmer when deep plowing and it favors the bacteria life cycle.

Firstly, Jae-JinLee et al. published the low-level radiation resistance of the genus of Flavisolibacter[18]. The proportion of Flavisolibacter in the southern virgin soil was 1.26%, the use of farming systems contributed to an increase in its proportion to 1.55% at no-till and to 1.97% at TFS. The effect of introduced rhizobacteria depended on the farming system. At no-till, there was a slight increase in their proportion and a decrease at TFS. Analogous tendencies were observed with minor
members of the genus of *Burkholderia*. The genera *Burkholderia*, *Flavisolibacter* and *Pseudomonas* were noted among rhizosphere bacteria reacting to maize plant development [19].

### Table 1. The proportion of genera in the prokaryotic biome of southern chernozem in the application of CMPs and different farming systems (with the highest level of intergroup coefficients of principal components)

| Phylum           | Family               | Genus               | No-till without CMPs | No-till with CMPs | Traditional system without CMPs | Traditional system with CMPs | Virgin soil |
|------------------|----------------------|---------------------|----------------------|-------------------|---------------------------------|-------------------------------|--------------|
| Crenarchaeota    | Nitrososphaerae      | Candidatus nitrososphaera | 6.21                | 7.47              | 9.20                            | 9.72                          | 7.34         |
| Actinobacteria   | Rubrobacterae        | Rubrobacter         | 2.71                | 2.84              | 3.55                            | 3.52                          | 3.10         |
| Bacteroidetes    | Chitinophagaean      | Flavisolibacter     | 1.55                | 1.71              | 1.98                            | 1.69                          | 1.26         |
| Firmicutes       | Bacillaceae          | Bacillus            | 0.68                | 0.90              | 1.84                            | 0.88                          | 0.68         |
| Proteobacteria   | Bradyrhizobiaceae    | Skermanella         | 1.84                | 1.91              | 2.65                            | 2.98                          | 1.85         |
| Verrucomycota    | Rhodospirillaceae    | DA101               | 1.31                | 1.16              | 1.87                            | 1.98                          | 1.84         |

The sum of the fractions: 18.78, 20.89, 25.55, 24.47, 19.17

Bacteria from the genus of *Segetibacter* are gram-negative, strictly aerobic heterotrophs. And, naturally, the traditional farming system enhancing soil aeration contributed to an increase in the proportion of these microorganisms by 2.7 times compared to virgin soil, as well as with no-till. Using biopreparations the proportion of *Segetibacter* genus decreased by 2.1 times in TFS and increased by 1.3 times in no-till.

Among the dominant Alphaproteobacteria, two genera *Balneimonas* and *Skermanella* have been noted. The genus of *Balneimomas* belonging to the family of *Bradyrhizobiaceae* in the condition of TFS was at the same level as in the case of virgin soil (1.87 and 1.84 %), at no-till – 1.4 times lower. The proportion of representatives of *Skermanella* of the family of *Rhodospirillaceae* was the maximum at TFS and amounted to 1.89%, which is 1.4 times higher than virgin soils and 1.7 times no-till. The use of CMPs has contributed to a slight increase or decrease in the proportion of these genera depending on the farming system.

It should be noted that among the dominant unattributed genera of Actinobacteria, the proportion of ones from the family *Gaiellaceae* exceeded 5.0% in virgin soil.

Eurybiont microorganisms that can tolerate changes in the environment include the genera *Bacillus* and *Pseudomonas*, among which there are both phytopathogenic species and their antagonists, which are widely used in biotechnology. The genus *Bacillus* combines bacteria that form endospores resistant to chemical, physical agents and pathogens [20, 21]. The representatives of this genus also prefer more aerated soils. Therefore, the number of them in the southern chernozem of our stationary was also the highest in the TFS, which is 1.4 times higher than virgin soil and no-till. There are tendencies to increase their proportion under the influence of CMP in both farming systems. The proportion of *Pseudomonas* in stationary samples was higher than that of virgin soil (0.02%). In the conditions of direct seeding, the excess was 6.1 times, in TFS was 1.4 times. The use of the CMPs has contributed to a slight increase in their representation.
The representatives of the genera *Erwinia* and *Xanthomonas* include widespread phytopathogenic species. In the conditions of southern chernozem, they were among minor representatives. The proportion of the genus *Erwinia* was critical among the studied samples in virgin soil (0.014%), 2.3 times less at no-till and 4.7 times at TFS. The use of CMPs had provided for its increase, especially in no-till to 2.2 times. *Xanthomonas* is only detected at no-till without CMP.

*Cellulomonas*-secreted multidomain proteins participate in cellulose decomposition [22]. In southern chernozem, the highest proportion of this genus is found in virgin soil (0.553%), while farming systems have contributed to its decline: to 1.6 times at no-till and to 1.3 times at TFS. In the application of CMPs, there were also tendencies towards a decrease in the representation of *Cellulomonas* in both farming systems. It should be noted that according to the direct sowing, the decline was more significant and amounted to 1.5 times.

A distinctive feature of the genus *Rhodococcus* is their metabolic plasticity, which ensures their wide distribution in nature. Having one of the largest known bacterial genomes, this genus forms a huge reservoir of secondary metabolites and is involved in the transformation of ecologically important organic compounds [23]. In the studied samples of southern chernozem, the genus *Rhodococcus* was found among minor representatives at the genus level with a maximum proportion in virgin soil (0.027%). Its decrease was noted in the conditions of both farming systems: to 2.3 times at no-till and to 6.7 times at TFS. The use of complex microbial preparations contributed to the reduction of their representation.

The representatives of the phototrophic genus of bacteria *Rhodoplanes* and symbiotic nitrogen fixator *Bradyrhizobium* are more common in soils under the forest than in agrocenoses. In the conditions of southern chernozem under the influence of the direct sowing technology, their proportion was maximum and amounted to 0.906 and 0.018%, respectively. Their representation in the samples is not related to the content of available forms of nitrogen, the indicators of which were the lowest in virgin soil, as well as the representation of these genera. The proportion of *Rhodoplanes* declined in the conditions of the traditional farming system by 1.9 times and virgin lands by 2.1 times. A 2.0-fold decrease in *Bradyrhizobium* representation was also observed in TFS, whereas it was not found in virgin soil.

In the management of the organic farming system, there is an increase in representatives of the genus *Hylemonella*. In the studied stationary at no-till with CMPs, their proportion was at the level of virgin soil, whereas at TFS it is 6 times less, with CMP the gap is reduced to 1.5 times.

The genus *Methylotenera* participates in the transformation of organic matter introduced into the soil using methane and its derivatives [24]. In southern chernozem, its maximum proportion was noted at no-till with CMPs, at TFS with CMPs it is less than 3 times, and in virgin soil, it was not found.

**Table 2.** The influence of farming systems and CMPs on the abundance of genera of the prokaryotic biome of southern chernozem (%)

|                      | No-till          | Traditional system | Virgin soil |
|----------------------|------------------|--------------------|-------------|
|                       | without CMPs     | with CMPs          | without CMPs | with CMPs | without CMPs | with CMPs |
| Known species        | 37.448           | 39.053             | 40.537      | 40.047    | 35.121       |
| Non-attributed species | 62.552          | 60.947             | 59.463      | 59.953    | 64.879       |

The representatives of the genus DA101, belonging to the family *Chthoniobacteraceae* and the phylum *Verrucomicrobia* are widespread in soils of meadows [25]. The conditions of southern chernozem under the influence of the direct sowing provided their maximum proportion of 1.54%, which is 4.3 times higher than the traditional farming system and to 6.2 times of virgin soil. The introduction of microbial preparations contributed to an increase in the representation of the genus DA101 by 1.2 times in the direct sowing and a decrease by 1.8 times in TFS.

All the above-described genera of microorganisms are known for their ability to grow in artificial conditions, which contributed to the study of their physiological properties. According to the data
obtained by the high-performance sequencing, the unattributed genera with values from 59.5 to 64.9% (490 OTU) for each of the variants had a greater total proportion compared to the genera with known taxonomy, which in the future requires a more detailed study (table 2).

4. Conclusion
The taxonomic structure of the microbiome of southern chernozem compared to virgin soil was studied in the stationary experiment on the use of microbial preparations in the conditions of traditional for the steppe part of the Crimea and each culture of the five-field crop rotation of the farming system and no-till. In the prokaryotic biome of southern Chernozem 823 species of microorganisms have been identified. According to the analysis of the main components, 7 dominant genera were revealed: Candidatus Nitrososphaera, Rubrobacter, Flavisolibacter, Segetibacter, Bacillus, Balneimonas, Skermanella. Microorganisms from the genera Erwinia and Xanthomonas with phytopathogenic properties were found with a minimum proportion in communities from 0.003 % to 0.014 % and 0 – 0.005%, respectively. The low level of cellulose-decomposing bacteria of the genus Cellulomonas was noted in southern chernozem. The critical proportion of this genus is found in virgin soil (0.55%), while the farming systems have contributed to its decline: to 1.6 times at no-till and to 1.3 times at TFS.

Thus, the metagenomic analysis of the taxonomic composition of the soil microbiome with the use of high-performance sequencing showed that the use of microbial preparations reduces the negative impact of the no-till farming system on the microbiocenosis of southern chernozem and improves its environmental feasibility in the Crimean steppe.

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