

AGRICULTURAL SCIENCES

ESTIMATION OF STRUCTURAL-SPATIAL ORGANIZATION OF BIOME IN AGROECOSYSTEMS

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DOI: <https://doi.org/10.30525/978-9934-26-050-6-8>

Balance of vital activity of the soil microbial complex is an important condition that determines the soil preservation and ecological balance of agroecosystems [1, p. 86]. Modern agrotechnogenic load disrupts natural processes in agrophytocenoses and leads to a decrease in the fertility level and imbalance of microbiological processes in the soil [2, p. 25]. Comprehensive research of soil microbial biome is necessary to understand and disclose the mechanisms in the system «soil – microorganisms – plant» is to create sustainable and highly productive agroecosystems [3, p. 68]. At present, the assessment of the structural and spatial organization of biomes in agroecosystems by a complex combination of classical and molecular biological methods has become particularly relevant and determines the purpose of the research.

The research was carried out based on the stationary field experiment of the National University of Life and Environmental Science of Ukraine in the Forest-Steppe zone in grain-beet 10-field crop rotation. The influence of agrarian systems against the background of differentiated tillage on the microbial biome of typical chernozem was studied in the main phases of the sugar beet ontogenesis (germination, leaves closing in-row spacing, and full maturity). The intensive system of agriculture (control) provided for the application of 12 tons of manure and mineral fertilizers per 1 ha of crop rotation area at the rate of $N_{92}P_{100}K_{108}$, the ecological system – 24 t/ha of organic fertilizers and mineral fertilizers at the rate of $N_{46}P_{49}K_{55}$, the biological system provided the application of 24 t/ha of organic fertilizers.

The number of bacteria, micromycetes, and actinomycetes was determined by the inoculation of soil suspensions on agar nutrient media [4, p. 4]. The content of active microbial biomass was evaluated by the substrate-induced respiration method [5, p. 179]. The microbial metabolic coefficient was

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determined by calculation. The structure of the qualitative composition of soil microorganisms was studied by generally accepted methods for morphological and cultural properties [4, p. 13]. The diversity of soil microbial complexes was assessed by the ecological indices of Shannon and Simpson [6, p. 147]. The taxonomic structure of prokaryotes of typical chernozem was determined by the pyrosequencing method [7, p. 212].

The ratio and number of researched groups of microorganisms depend on the phenophase of plants, and applied agricultural measures have been established. In the germination phase of sugar beet, the number of soil microbiota was the lowest and was: bacteria – 6.13-9.41 million CFU/g of soil, micromycetes – 21.22-24.63 thousand, actinomycetes – 0.79-0.97 million. The intensity production of root exudates increased with the growth and development of plants, which contributed to the increase in the number of microorganisms in typical chernozem. Thus, the number of bacteria during the sugar beet ontogenesis increased by 1.3-2.7 times, while micromycetes – in the phase of leaves closing in-row spacing – decreased by 12.1-34.7 %, and in the phase of full maturity, on the contrary, increased 2.1-3.5 times. As for the actinomycetes number, such a pattern was not found. This indicates a different intensity of the conversion of organic compounds in the soil. The application of biological and ecological agrarian systems, compared to intensive, contributed to the growth of the number of studied groups of microorganisms during sugar beet ontogeny by an average of 14.6 and 18.6 %. But in the phases of leaves closing in-row spacing and full maturity, the number of micromycetes was increased at the intensive agriculture by 17.7 and 27.1 %, respectively.

It should be noted that it was a high correlation level ($r = 0.77$) between the number of bacterial and fungal microbiota during the entire ontogenesis of the culture for all agricultural measures. As for the number of actinomycetes, such a pattern did not found. The correlation level between the studied groups of microorganisms was high ($r = 0.66-0.97$) for all agricultural measures only in the germination phase.

The content of total (bacterial + fungal) active microbial biomass during the sugar beet ontogenesis ranged from 168.3–231.0 mg C/kg. The main share (78.7–88.9 %) of biomass was occupied by bacteria, while the share of micromycetes was much smaller and amounted to 11.1–21.3 %. The intensity of root secretions increased with the growth and development of plants. It led to an increase in the content of active microbial biomass in the middle and at the end of the culture ontogeny by 8.3-21.6 and 9.0-28.5 %, respectively. It has established that the content of bacterial biomass (including total) increased with a decreasing rate of mineral fertilizers in the direction: intensive → ecological (by 11.0 %) → biological (by 19.4 %) agrarian

system. It indicates an inhibitory influence of mineral nutrients on the metabolic properties of microorganisms. However, the biomass content of micromycetes in this direction, on the contrary, decreased (by 13.5 and 19.5 %, respectively).

It should be noted that the correlation level between the content of total active microbial biomass and the number of bacteria ($r = 0.80$) was high during the sugar beet ontogenesis. It indicated an increase in the amount of microbial biomass with the increasing of microorganism's number in typical chernozem.

The indicators of the microbial metabolic coefficient during the sugar beet ontogenesis were 0.30–0.37. At the same time, the stability of soil microbiocenosis during the culture ontogenesis increased with decreasing dose of mineral fertilizers in the direction: intensive → ecological (by 3.8 %) → biological (by 19.7 %) agrarian system.

Analysis of the qualitative composition of the typical chernozem microbiota based on the description of morphological and cultural properties of bacterial and micromycetes colonies shown that the studied microbial complexes differ among themselves in the number of detected morphotypes and the structure of distribution of dominant forms of microorganisms. Thus, the number of detected morphotypes of bacteria depending on the experiment variant was varied between 26–44 CFU, micromycetes – 16–36 CFU. The share of dominants among them was 2.3–11.8 %, subdominants – 2.8–33.3 %, representatives of soil microbiome that often occurs – 18.8–94.4 %. The use of agricultural measures has created the conditions for redistribution of the relationship between taxonomic groups of microorganisms due to representatives, that often occurs, as well as the degree of presence of active forms of random species that are not permanent components of the microbial complex and are activated only in the presence of easily digestible nutrients in the soil.

It was found that the dominant forms of prokaryotes on morphological and physiological characteristics belonged to bacteria of the genus *Achromobacter*, *Pseudomonas*, *Bacillus*, *Micrococcus*, and actinomycetes *Nocardia* and *Streptomyces* with a saturation of 11.2–18.4 % depending on the applied agricultural measures.

The ecological indices of Shannon (I_{Sh}) and Simpson (I_S) indicated a high diversity of bacteria ($I_{Sh} = 1.23$ – 1.43), their uniform distribution ($I_S = 0.05$ – 0.07) during the sugar beet ontogenesis at all agrarian systems. The micromycetes diversity was slightly lower ($I_{Sh} = 0.83$ – 1.50), and it was characterized by an uneven distribution ($I_S = 0.04$ – 0.30) of morphotypes. The application of biological and ecological farming has contributed to the growth

of polyfunctionality and diversity of bacteria and micromycetes comparatively intensive.

Analysis of the prokaryotes metagenome by pyrosequencing revealed 367 taxa, of which 22.3% belonged to unidentified sequences. Among the identified taxa, 25 were bacteria, and 2 were archaea. At the genus level, the dominant representatives among the identified prokaryotes were representatives of *Achromobacter* (36.6-37.5 %) and *Pseudomonas* (21.4-29.0 %), subdominants were *Candidatus Nitrososphaera* (2.6-2.7%), *Pseudonocardia*, *Thermomonas*, *Bacillus*, *Rubrobacter*, *Streptomyces*, *Mycobacterium*, *Candidatus Solibacter*, *A4*, *Hyphomicrobium* (0.1-0.4%), etc. The biological agrarian system was characterized by the greatest microbial diversity at the genus level, and intensive led to the impoverishment of the polymorphism of prokaryotes genetic resources of typical chernozem.

The species richness of microorganisms in the sugar beet rhizosphere, according to the ecological indices of Shannon ($I_{Sh} = 4.2-4.88$) and Simpson ($I_S = 0.80-0.83$), obtained by pyrosequencing, was significantly higher than the results methods of classical microbiology has been found. So, the use of molecular biological research methods makes it possible to investigate to a greater extent the structure of microbial diversity, mainly due to non-cultivated forms.

Thus, the microbiome research by classical and molecular biological methods of analysis shows that the systematic application of organic fertilizers to the soil (biological agrarian system) contributes to the formation of optimal functioning of the soil microbial complex, accompanied by increasing numbers, diversity, stability and biomass content of microorganisms in typical chernozem during the sugar beet ontogenesis. The conditions of soil microbiota functioning deteriorated with the priority application of mineral fertilizers (intensive system).

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