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TAXONOMIC COMPOSITION AND ORGANIZATION OF THE MICROBIAL COMMUNITY OF SODDY-PODZOLIC SOILS AFTER APPLICATION OF STRAW OF CEREAL CROPS AND USING OF THE BARKON BIOPREPARATION

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Abstract

The modern concept of the reproduction of soil organic matter (SOM) requires the sequestration of the carbon of plant residues in the soil by the formation of stable organic compounds. In this regard, the role of microbial preparations, accelerating the decomposition of straw are important. Learning the taxonomic structure of the microbial community in these processes is of great importance and not well understood. Microbial communities of arable soddy-podzolic soils decomposing straw of grain crops were studied in field and laboratory experiments. Straw (rye, wheat and oat) were crushed and inoculated with the Barkon preparation (complex association of microorganisms developed at the FGBNU ARRIAM). The functioning of the microbial community was assessed by the number and activity of microorganisms, the agrochemical properties of the soil. The composition of bacterial community of soils was determined by high-performance sequencing of 16s rRNA gene libraries. The rate of decomposition of straw was controlled by the ratio C:N in it: rye straw < wheat < oat. Barkon increased rate of decomposition of straw by 18-42% compared to soil microflora by 3 months of composting. Biopreparation is more effective when straw is incorporation in the 0-5 cm layer than by 9-12 cm. The effect of the Barkon on the number, biomass of microorganisms, and their respiration was not noticeable as compared with the growth of these parameters when introducing straw. The absence of an increase in carbon dioxide emissions with an increase in the rate of straw decomposition when Barkon is introduced, indicates an intensification of the processes of carbon sequestration in soil. The treatment with a biological preparation promotes the formation of microbial destructive communities with the highest efficiency of straw conversion and its conversion into labile organic compounds, and then into soil humus substances. Therefore, the use of Barkon, compared to the uninoculated straw, increased the content of total carbons in the soil by 4.8 to 8.4%. All studied factors (soil, straw, biological preparation, depth and time of decomposition) influence on the composition of microbial communities leading decomposition, the most significant of which is the type of soil. This confirms the high response of the composition of the microbial community to various factors while maintaining the crustal component of the microbiome characteristic of this soil. In the more acid soddy-podzolic soils, at the same humus content, in the taxon *Acidobacteria*, group 1 and group 2 prevailed, while in the soils with a neutral pH, group 6 predominated. The indicator of straw

application for sod-podzolic soil in all experiments is the increase of *Actinobacteria* from the family *Micrococcaceae*, particularly in variants with straw inoculation with Barkon, since *Micrococcaceae* is one of the microbial components of this biopreparation. Detected the influence of adding straw and application of the Barkon on the taxonomic composition of the bacterial community and the configuration of the destructive biosystem of soil microorganisms tuned for humification of plant residues. The decomposition of straw in the soil, as compared to that which was not planted, showed some weakening of Barkon's effect on the formation of the humification trophic chain, as evidenced by the lack of growth of labile humic substances in the respective variants. Based on the extended taxonomic data on the composition of the soil microbial community, it was found that minor groups of microorganisms participate equally with major groups, forming network fractal structures.

Keywords: microbial community, straw, a microbiological preparations, Barkon, the index of fractal structures

The decomposition of plant residues and the formation of organic matter (humus) resistant to microorganisms in arable soils play an important role in the global carbon cycle in the biosphere. The use of straw as an organic fertilizer becomes essential in the world, especially in connection with the developed no-till technology [1-5]. For example, due to the sharp reduction in Russia of the resources required for traditional organic fertilizers, the need for them in order to reproduce the soil humus can be satisfied only by 17-20%. The positive effect of the introduction of straw on the nutrient regime, the physical state of the soil, the processes of humus formation, the number and activity of soil microorganisms [2, 3, 5-7] is known. However, straw is used insufficiently because of its long-term decomposition, which is accompanied by a deficit of mineral nitrogen in the soil, released phytotoxic compounds and accumulated phytopathogens. The solution can be the straw treatment with biopreparations to accelerate its decomposition and eliminate possible negative consequences, which is especially important for the no-till technology [8-10]. Only 10-20% of straw with no treatment with special microorganisms is converted into humus when introduced into the soil. However, the modern concept of the reproduction of soil organic matter (SOM) involves the fixation of organic carbon in soil (sequestration) with a decrease in their mineralization to CO₂ [1, 11], so it is important that biopreparations for straw biodegradation enhance the inclusion of crop residue carbon into soil organic matter [2, 6, 12].

Management of the soil biological component to increase the production of agricultural products becomes one of the urgent tasks under the conditions of unstable climate and increasing soil degradation, since the possibilities of chemicals and "green revolution" reduced [12]. The significance of biodiversity and the total activity of microorganisms for the soil functioning are generally well studied, but the effect of the presence and ratio of certain species and genera of microorganisms (taxonomic structure) on biological processes, as well as self-organization of the microbial community in a particular soil difference [13-16] have not been studied sufficiently.

Studying the patterns of formation of the microbial community structure and its relationship with the properties and functioning of the soil is one of the most important areas of modern science [12-14, 16, 17]. The task is complicated by the so-called redundancy of the microbial system, when one function (cellulose decomposition, nitrogen fixation, denitrification) is performed by many microorganisms. A decrease in the number or absence of some species of microorganisms may not affect the general nature of the process in the soil (a particular soil function), since their role is shifted to others [13]. For this reason, the composition of the soil microbial community is not determined when assessing the soil nutrient regime, calculating the planned yield, or modeling large-scale cycles under stable conditions [13, 14]. However, at present, when abrupt climate changes during the season (abnormal heat or cold, drought or excess rainfall),

together with the soil degradation, are becoming the norm, information on the composition and structure of the microbial community can reduce the uncertainty of predictions regarding the result of plant residues biodegradation and even the yield forecast [13, 14, 16].

The decomposition of plant residues in soil is determined by many factors (chemical composition, soil physical properties, and hydrothermal conditions); therefore, significant differences are observed in the process speed, the quality of final products, humification coefficients and other indicators [1, 18-20]. Currently, there are three main hypotheses about the effect of biological factors and chemical composition on the final result of plant residues biotransformation [18]. According to the chemical hypothesis, regardless of the initial composition of residues and microbial destruction community, the same compounds will be obtained at the final stage of transformation (for example, humus of the same type). According to another hypothesis, various humic substances can be obtained depending on the initial chemical composition of plant residues. According to the third hypothesis, compounds are formed depending on the composition of the microbial community responsible for decomposition.

One of the ways to control the composition of the microbial community that decomposes straw is the introduction of biopreparations. Introduced microorganisms often work only at the initial stage, and then their number decreases, and the microbiocenosis returns to its original state [21, 22]. However, there is an option when changes in the microbial community composition after introducing biopreparation are maintained until the end of decomposition. The latter is possible not only as a result of direct exposure to the number of introduced microorganisms but also due to the ability of biopreparation to change the connections between them while making trophic chains in a new way. In both cases, it is possible to obtain substrates that differ quantitatively and qualitatively at the output. This issue has not been studied sufficiently, although it is very important for assessing the trend of soil-biological processes and the importance of the repeated introduction of biopreparation.

The Barkon preparation (All-Russian Research Institute of Agricultural Microbiology) is an association of microorganisms that capable of destruction of lignocellulosic substrates and their subsequent transformation into humic substances [23]. The preparation has a stimulating effect on the microbiological processes of straw transformation via increasing the number of microorganisms, microbial biomass, the coefficient of straw humification, and eliminating phytotoxicity [6, 23-25]. When processing the straw (stubble) with Barkon without embedding it in the soil, microorganisms participating in plant residues transformation form a humified trophic chain, providing more effective incorporation of both introduced and native microorganisms into humus-forming processes.

The present study for the first time establishes that straw inoculation with Barkon contributes to the formation of destructive microbial biosystems with a set of the most effective microorganisms from the soil community and organized action on the straw residues decomposition. It is shown that soil properties, rather than biopreparation, the type or depth of straw embedment, have the greatest influence on the composition of communities of microbial destructors of straw.

The purpose of the paper was to assess the taxonomic composition of the soil microbial community which decomposes the straw of cereals, and the role of microorganisms of Barkon biopreparation in the change of the soil microbiome.

Techniques. The composition and functioning of the microbial community of arable sod-podzolic soils when introducing straw of cereals inoculated with the Barkon biopreparation [23] was studied in lab and field experiments. The straw chopped into 1-2 cm pieces was treated with the preparation according to

the developer's recommendations (1 ml of the preparation + 25 ml of water per 10 g of dry straw). The control was options without straw (absolute control) and with straw treated with water.

In lab test 1, soft wheat straw ($3.5 \pm 0.2\%$ ash, $0.7 \pm 0.03\%$ N, C:N = 69) was inoculated with the Barkon biopreparation or soil suspension (10 g of soil in 90 ml of water) and composted outside the soil for 1 month. The composted straw was introduced into the soil collected from the arable horizon of sod-podzolic soil (Leningrad Province, settlement Belogorka; $S_{\text{hum.}} 1.27 \pm 0.02\%$, $N_{\text{total}} 0.11 \pm 0.003\%$, $\text{pH}_{\text{straw}} 4.92 \pm 0.03$) at a rate of 3 g/kg soil and stirring evenly. The experiment was carried out in 250 ml glass vessels at a constant humidity of 60% FMC (field moisture capacity) and a temperature of 25 ± 2 °C. The duration of composting with the soil was 3 months, with 2 vessels per each term of estimation and 3 vessels for the final estimation.

In lab test 2, plastic 1.5-liter pots were filled with cultivated sod-podzolic soil from the arable horizon (St. Petersburg—Pushkin, Detskoselsky State Farm; $S_{\text{hum.}} 4.02 \pm 0.06\%$, $N_{\text{total}} 0.316 \pm 0.02\%$, $\text{pH}_{\text{straw}} 5.6 \pm 0.01$). Rye straw ($3.4 \pm 0.04\%$ ash, $0.25 \pm 0.02\%$ N, C:N = 193) was treated with Barkon or water and, after 1 h, added to pots at a rate of 3 g/kg soil. Two variants for embedding straw were studied, surface (0-3 cm) and deep (9-12 cm). The experiment was arranged in 5 replications 5, the test lasted 62 days at a constant humidity of 60% FMC and 25 ± 2 °C. The paper presents data for 5 variants out of 13.

In the field experiment 3, oat straw (4.9% ash, $1.40 \pm 0.01\%$ N, C:N = 34) was treated with Barkon or water, mixed with soil ($S_{\text{hum.}} 1.96\%$, $N_{\text{total}} 0.194\%$, $\text{pH}_{\text{straw}} 5.62$) at the rate of 3 g/kg, then placed in nylon bags and put in the soil to a depth of 0-5 and 10-15 cm (experimental field of the All-Russian Research Institute of Agricultural Microbiology, Pushkin). The experiment lasted for 1 month and was arranged in 9 replications.

Analyses of straw and soil (a mixed sample) were performed in 3-5 replications using standard methods [26, 27]. The content of undecomposed straw in the soil was determined by flotation in 0.5 normal Na_2SO_4 [28], the amount of total carbon by wet ashing with potassium bichromate, labile water-soluble organic carbon by the method of Schulz [29]. Movable humus compounds were isolated from soil by 0.1 normal Na-pyrophosphate (pH 7.0 or 10.0). The carbon content in the extracts was evaluated at $\lambda = 340$ nm (Ultraspec spectrophotometer, LKB, Sweden) [30]. Soil respiration was measured with a Tsvet 110 gas chromatograph (OAO Tsvet, Russia; the katharometer was a detector, and the gas carrier was helium). Microbial biomass in the soil was determined by substrate-induced respiration [31] as total (fungi + bacteria) and fungal biomass (treatment with streptomycin and rifampicin, 16 mg of antibiotic per 1 g of soil). In experiment 1, only fungal biomass was determined; in experiment 3, only the number of fungi was determined. Nitrogen and carbon of the microbial biomass were estimated using the rehydration method [32]. The number of physiological groups of microorganisms (ammonifying, amylolytic, cellulose-decomposing, humus-decomposing, micromycetes) was counted on dense nutrient media by soil suspension-plating method [33].

The structure of the soil bacterial community was determined using high-throughput sequencing of the 16S rRNA gene libraries for individual periods: 2 months for laboratory experiments (without replications, from mixed samples according to variants), 3 and 17 days in a layer of 0-5 cm for a field experiment (repeated 3 times).

The taxonomic composition of the bacterial community was determined using high-throughput sequencing of the 16S rRNA gene libraries. For this,

DNA was isolated from soil using MoBio kits (Qiagen, Germany) [34], libraries were produced by PCR with universal primers F515 and R806 for 16S rRNA gene [35]. Sequencing was performed using a GS Junior instrument (Roche, USA); the results were processed in the QIIME program [36].

The biodiversity of microbial communities was assessed according to the Shannon diversity index and the Sørensen-Czekanowski coefficient of similarity. Processing with Statistica v6 software (StatSoft, Inc., USA) involved standard methods of multidimensional statistics, i.e. principal component analysis, dispersion, correlation, fractal analysis, and graph analysis. The necessary calculations were performed using dispersion, correlation and fractal analyses [37-40] using original computer programs. Tables and text show mean values (M) with confidence intervals at $p \leq 0.05$ significance level ($t_{0.05} \times \text{SEM}$). For comparison of libraries (with small frequencies), the probability that the frequency of membership in a taxon will be the same for two libraries was estimated [41].

The probability of the observed difference (significance) in the assignment to taxon T was estimated by the formula:

$$p(y|x) = \left(\frac{N_2}{N_1}\right)^y \frac{(x+y)!}{x!y! \left(1 + \frac{N_2}{N_1}\right)^{(x+y+1)}}$$

where N_1 and N_2 are the total number of sequences for libraries 1 and 2, x and y are the number of sequences assigned to T, for libraries 1 and 2, respectively. One of the fundamental assumptions for this formula is that x and y are relatively small compared to N_1 and N_2 (less than 5% of the total), and N_1 and N_2 are relatively large (more than 500).

Results. Since the relationship between the nitrogen content in plant residues and the rate of their biotransformation [44, 43] is well known, it is likely that significant differences in the loss of straw in different experiments are related to the nitrogen content in it. The effect of the microbial community composition on the rate of biodegradation is unlikely: a decrease in mass for 1 month for wheat straw (experiment 1) under constant hydrothermal conditions was 2.6-3.5%; for oat straw in field conditions (experiment 3) this was from 20.0 to 31.8% at similar values of the total carbon content in the soil. Rye straw decomposed most slowly (experiment 2): for 2 months its weight loss was comparable to that for 1 month for wheat.

For 1 month of composting soil with straw, the use of Barkon did not have a statistically significant effect on the rate of straw decomposition, regardless of its type, depth of embedment and soil fertility. Decomposition under the influence of the biopreparation was significantly accelerated only at the beginning of month 3 (experiments 1 and 2): the weight loss of straw was 18-42% higher compared to the native soil microflora. Experiment 2 identified the efficacy of Barkon in biodegradation in the upper soil layers: the decrease in straw in the 0-3 cm layer was 14.8% more, and in the 9-12 cm layer was 7.6% less than with the native microflora. Decomposition of straw in the field also proceeded somewhat faster in a layer of 0-5 cm (a straw decrease of 30.8-31.8%) than in the lower layer (20.0-30.0%). The reason for the lower efficiency of the deep embedding of straw inoculated with Barkon may be that the preparation contains aerobic microorganisms [23, 24]. When creating facultatively anaerobic conditions in the lower layers (in separate niches), including that resulting from the activity of microorganisms, the effect of Barkon is worse, and the change in the trophic relationships of the microbial community even worsens the situation regarding the variant without biopreparation.

In experiment 1 with preliminary composting of wheat straw, the use of Barkon for 30 days increased the formation of water-soluble humus-like com-

pounds compared to the variant with native microflora: the absorption index was 9.0 ± 0.2 vs. 8.0 ± 0.1 , E_{465}/E_{665} coefficient (humus content) 2.7 ± 0.0 vs. 1.9 ± 0.4 . It should be noted that both in control and under the action of Barkon, the ash content changed equally (from 3.5 ± 0.2 to $5.3 \pm 0.6\%$), as well as the content of nitrogen (from 0.7 ± 0.05 to $1.07 \pm 0.08\%$), phosphorus (from 0.06 ± 0.0 to $0.16 \pm 0.03\%$), and the C:N ratio (from 69 up to 28). It is the influence of Barkon during the preliminary composting that can be associated with a 25.6% increase in the content of newly formed labile humic substances in experiment 1 as compared to their amount when using soil inoculum (Table 1). In the variant straw + Barkon, humus-like compounds were formed when decomposing (see increase in C_{lab}) which are probably less accessible to microorganisms. This led to an increase in the total carbon content in the soil. On the contrary, in the variant straw + soil inoculum, microorganisms increased by 67.4% (Table 2) and after dying out, because of low C:N ratio (3.4-5.8), were less included into humus. In addition, in this variant, humus was even lower compared to the control, due to the fact that either the microbial community strenuously decomposed not only straw but also labile humic substances, or they were formed less. Since the biomass of microorganisms was characterized by low C:N, humic substances probably contained more nitrogen, which resulted in an increase in the content of total nitrogen in the soil.

1. Agrochemical parameters of sod-podzolic soil when introducing straw and using Barkon biopreparation ($M \pm t_{0.05} \times SEM$)

Variant	C_{total} , %	N_{total} , %	Total N-mineral, mg/kg	C-CO ₂ , mg · kg ⁻¹ · day ⁻¹	C-labile hu- mus, mg/kg
	в конце опыта			среднее за опыт	
Lab experiment 1					
Control	1.24±0.02	0.106±0.003	48.7±3.0	8.6±1.3	699±25
Straw + soil inoculum	1.25±0.05	0.111±0.001	19.8±0.2	23.6±1.8	636±51
Straw + Barkon	1.48±0.06	0.106±0.005	25.7±1.0	25.8±0.9	737±55
Lab experiment 2					
Control	3.78±0.05	0.313±0.001	26.9±0.8	8.8±0.1	7250±120
Straw, 0-3 cm	3.83±0.04	0.310±0.001	22.4±0.8	17.7±1.6	8110±320
Straw, 9-12 cm	3.81±0.14	0.306±0.010	18.1±1.7	13.8±0.8	7620±280
Straw + Barkon, 0-3 cm	3.84±0.03	0.305±0.010	22.9±0.0	20.7±1.9	6790±10
Straw + Barkon, 9-12 cm	3.89±0.08	0.307±0.010	21.8±1.5	15.2±3.2	7040±500
Field experiment 3					
Control, 0-5 cm	1.85±0.05	0.178±0.001	15.3±0.1	8.4±1.8	1354±5
Control, 10-15 cm	1.88±0.01	0.178±0.001	24.6±1.0	6.6±1.0	1335±22
Straw, 0-5 cm	1.98±0.05	0.187±0.002	15.3±0.1	22.6±3.9	1403±7
Straw, 10-15 cm	1.89±0.09	0.167±0.002	17.8±1.0	27.4±3.7	1413±9
Straw + Barkon, 0-5 cm	2.13±0.08	0.179±0.001	19.5±0.1	30.0±2.3	1369±14
Straw + Barkon, 10-15 cm	1.98±0.08	0.182±0.002	24.6±2.0	25.3±2.0	1357±22

Note. For a description of the experiments, see the *Techniques* section. Labile humus — C content in 0.1 normal pyrophosphate extract; pH 7.0 for experiments 1 and 2, pH 10.0 for experiment 3.

When embedding straw into the soil immediately after treatment with Barkon (experiments 2 and 3), no accumulation of newly formed humus compounds occurred (see Table 1), although it is impossible to make an unequivocal conclusion that this was not related to the type of straw. Nevertheless, the use of Barkon increases the content of total carbon in the soil compared to the variants without biopreparation due to the enhancement of straw transformation processes, including humification. For example, the complex index of humification [44] when introducing straw (experiment 2) was 2.87 in the control and 3.21 in the variant with biopreparation. Barkon somewhat weakened the negative effects of the straw introduction on the content of mineral forms of nitrogen in soils with a low content of organic matter (experiments 1 and 3). This can be considered as an advantage of Barkon, since its use does not require the obligatory introduction of mineral nitrogen for the straw decomposition [2, 3], which is especially valuable since usually biopreparation is used after harvesting grain crops

when nitrogen is not needed for plants.

The introduction of straw is expected to increase the respiration of microorganisms in all experiments (see Table 1). The influence of Barkon on this indicator vs. non-inoculated straw was insignificant. There was a tendency to an increase in the release of carbon dioxide from the soil only in experiment 2. Breathing enhancements were not observed in experiments with Barkon conducted by other researchers [6].

In the case of soils with a low total carbon content (experiments 1 and 3), the introduction of straw increased the number of the main groups of microorganisms 2 times or more (Table 2), which is consistent with the data on the greater efficiency of fertilizers and preparations on poor soils [2, 12, 45]. When introducing the straw, a short development of r-strategists occurs [2, 46]: in experiment 3 on day 3, there was an increase in the number of ammonifying and amylolytic microorganisms, which stopped on day 17. Treatment of straw with the preparation did not lead to significant changes in the number of cellulolytic microorganisms, except for experiment 2. In experiment 3, the influence of the biopreparation was even weaker and not permanent. So, on day 17, when introducing Barkon, a slight decrease in the number of cellulolytic microorganisms was in the 0-5 cm layer.

Barkon influenced the succession of microorganisms. Thus, in experiment 1, the coefficient correlation between the number of humus-decomposing microorganisms and the content of residual straw in the variants with Barkon and the soil inoculum changed its trend (-0.87 vs. 0.71). A significant influence on the strength and trend of the relationship between the dynamics of physiological groups and the amount of straw remaining in the soil was observed for other microorganisms: the correlation coefficients for the variants with the Barkon and without were 0.20 and -0.78 (cellulose-decomposing), -0.38 and 0.36 (amylolytic), respectively.

In all experiments, no significant effect of the introduction of straw, preparation and the depth of introduction on the number and/or biomass of fungi was found (see Table 2). Perhaps this is due to the fact that the role of bacteria increases in arable soils in the process of plant residues decomposition [45]. In the experiments, a certain increase in the number of bacteria occurred during the study, while the number of fungi varied slightly or decreased: their share of total biomass ranged from 14 to 22%. When introducing the straw, not only the number, but also the biomass of soil microorganisms increased, and the effect of treatment with biopreparation was insignificant (see Table 2). Under the action of Barkon, microbial biomass rather decreased, but its activity increased, it was expressed in the magnitude of nitrogen flows through biomass (serves as a function of the number and activity of microorganisms and reflects microorganisms work in the soil). The smaller size of the nitrogen flows in experiment 2 was caused by weak decomposition of rye straw and low nitrogen content in it, since the decomposition of straw in cultivated highly humus soil requires more available nitrogen [43].

The relationship between the structure of the microbial community and the agronomic properties of the soil during decomposition of straw follows from two tree diagrams [39] obtained for experiment 1, of which the first was drawn up according to microbiological (abundance, biomass, activity) and agrochemical indicators, and the second according to the taxonomic composition of the soil microbial community. Tree diagrams had an identical cluster structure (data not shown). The obtained results showed that the treatment with Barkon biopreparation had a strong effect on the microbial community structure, since this option was not included in one cluster with straw decomposed by soil inoculum.

2. Counts, content and activity of microbial biomass in sod-podzolic soil when introducing the straw and using Barkon biopreparation ($M \pm t_{0.05} \times SEM$)

Variant	Ammonifying, mln CFU/g soil	Amylolytic, mln CFU/g soil	Humus decomposing, mln CFU/g soil	Cellulose decomposing, thous. CFU/g soil	Micromycetes, thous. CFU/g soil	C _{m.b.} , mg/kg	N _{m.b.} , mg/kg	N flow, mg/kg
Lab experiment 1, 2 months)								
Control	3.4±0.2	3.1±0.1	1.4±0.1	0.95	—	344±4	55.3±12.2	54.0±4.1
Straw + soil inoculum	6.0±0.1	8.2±0.4	4.2±0.4	15	—	576±56	98.7±19.9	181.0±7.2
Straw + Barkon	6.8±0.2	7.8±0.8	4.4±0.4	15	—	344±52	102.0±9.3	25.0±3.4
Lab experiment 2, 2 months)								
Control	12.0±0.8	8.7±0.6	6.3±0.6	15.8±2.1	23.5±3.5	571±60	43.9±0	87.0±6.5
Straw, 0-3 cm	16.4±0.3	14.0±1.4	6.8±1.0	65.8±8.1	26.5±3.5	697±52	53.0±4.2	112.0±4.6
Straw, 9-12 cm	12.9±0.8	14.1±1.1	9.5±0.7	61.8±14.0	20.4±2.1	807±39	53.4±4.9	87.0±2.3
Straw + Barkon, 0-3 cm	14.3±0.5	13.4±1.5	8.6±1.0	127.2±16.1	30.4±3.7	626±34	45.9±2.7	129.0±2.1
Straw + Barkon, 9-12 cm	13.8±1.0	13.8±1.6	8.7±0.9	98.9±14.0	13.0±1.4	603±72	41.9±5.6	99.0±9.6
Field experiment 3, day 3/day 17)								
Control, 0-5 cm	18.0±5.9/19.6±7.9	9.2±2.8/9.2±4.5	4.3±0.2/5.5±1.3	8.0±2.8/10.4±2.3	13.6±4.2/18.8±4.2	562±1/410±12	28.8±0/35.2±0	253.0±2.3
Control, 10-15 cm	18.6±6.8/32.1±3.3	9.7±2.0/9.5±4.6	4.9±0.5/8.1±0.8	6.7±0.5/8.5±0.8	14.5±1.1/24.0±5.3	498±25/460±61	27.9±0/38.2±0	262.0±13.8
Straw, 0-5 cm	107.1±20.2/27.6±3.4	67.8±16.7/20.3±8.4	8.8±0.3/12.0±2.4	7.3±2.4/10.3±3.4	10.9±3.6/23.8±6.6	694±48/597±118	35.2±1.2/54.0±4.8	513.0±5.9
Straw, 10-15 cm	59.3±0.7/46.4±0	69.4±1.5/23.2±0	9.8±0.4/14.5±0.8	4.7±1.3/3.9±0.8	12.5±3.3/23.2±2.7	557±60/640±6	24.6±6.1/60.6±11	821.0±7.8
Straw + Barkon, 0-5 cm	70.6±2.1/46.8±12.1	54.1±3.5/21.1±5.6	11.7±0.5/12.2±1.7	11.3±0.8/8.2±1.3	16.5±3.2/27.6±2.3	525±61/694±14	27.2±0/54.0±4.8	758.0±4.9
Straw + Barkon, 10-15 cm	57.3±3.5/61.2±20.2	44.0±13.7/28.1±9.3	11.3±0.6/11.90	9.7±1.4/7.0±2.3	14.5±4.7/29.3±0	442±86/710±10	25.9±9.3/59.7±9.5	858.0±13.1

Note. For a description of the experiments, see the *Techniques* section. C_{m.b.} — C of microbial biomass, N_{m.b.} — N of microbial biomass. Dashes indicate that the indicator was not determined. The flow of nitrogen through the microbial biomass [32] was calculated for the entire time of the experiment: experiment 1 — 103 days, experiment — 92 days, experiment 3 — 31 days. Cellulose decomposing microorganisms in experiment 1 was determined by the method of limiting dilutions.

3. Distribution of bacteria in sod-podzolic soil according to major taxa (% of the total number of operating taxonomic units, OTU) when introducing straw and using the Barkon biopreparation ($M \pm t_{0.05} \times SEM$)

Variant	Major taxa (different rang)										I_F
	Archaea	Acidobacteria	Actinobacteria	Bacteroidetes	Chloroflexi	Firmicutes	Gemmatimonadetes	Planctomycetes	Proteobacteria	прочие	
Lab experiment 1											
Control	0.5	18.5	17.0	0.7	0.03	3.5	2.0	1.3	27.7	28.8	0.44±0.03
Straw + Barkon	1.3	23.7	12.6	1.8	0.10	1.8	1.8	1.6	32.8	22.5	0.75±0.03
Straw + soil inoculum	0.3	16.9	9.5	3.5	0.03	5.2	1.1	1.3	41.7	20.5	0.52±0.03
Lab experiment 2											
Control	1.0	4.1	24.5	0.3	3.5	3.9	3.6	1.2	55.5	2.4	0.60±0.03
Straw, 0-3 cm	1.4	5.4	26	0.5	3.7	3.1	3.8	1.9	51.1	3.1	0.58±0.03
Straw, 9-12 cm	1.1	3.6	32.4	0.8	4.2	4.6	3.6	2.6	44.2	2.9	0.62±0.04
Straw + Barkon, 0-3 cm	1.2	3.5	24.7	7.6	3.5	2.5	4.2	1.8	46.9	4.1	0.68±0.04
Straw + Barkon, 9-12 cm	1.1	4	28.9	0.7	4.5	3.5	4.8	1.0	49.0	2.5	0.58±0.04
Field experiment 3											
Control, 3 days	1.9	5.8	28.6	2.0	5.2	4.6	5.3	2.0	39.3	5.3	0.78±0.03
Straw, 3 days	1.7	5.5	30.7	2.6	5.5	4.7	4.2	2.3	37.6	5.2	0.88±0.03
Straw + Barkon, 3 days	2.4	5.3	34.9	3.8	4.9	5.1	4.1	2.2	32.8	4.5	0.79±0.03
Control, 17 days	2.7	6.8	28.7	2.9	6.2	6.3	5.8	2.5	32.5	5.6	0.83±0.03
Straw, 17 days	2.2	6.9	25.6	3.8	5.4	6.2	4.7	2.4	35.9	6.9	0.80±0.03
Straw + Barkon, 17 days	2.9	6.0	29.9	3.1	5.6	5.6	4.8	2.5	34.2	5.4	0.83±0.03

Note. For a description of the experiments, see the *Techniques* section. Archaea phyla, due to their relatively small number, are represented by the entire domain. I_F — the index of fractal structures, calculated using full taxonomy data (at a genera level).

The bacterial community members in the studied soils were basically representatives of the phyla *Actinobacteria* and *Proteobacteria*; a significant number of microorganisms belonged to *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes* (Table 3).

Barkon preparation contained a significant number of microbial species (Fig. 1), which did not coincide with the soil microbiome. For example, Barkon contains one order less typical soil inhabitants, the *Acidobacteria*. At the same time, the share of *Bacilli*, *Sphingobacteria*, and *Gemmatimonadetes* is higher in the biopreparation compared to the soil. The Barkon biopreparation is intended for the decomposition of cellulosic waste; therefore, there are many actinomycetes in its bacterial community (*Cellulomonas*, *Corynebacterium*, *Micrococccaeae*) which decompose cellulose and other hard-to-reach organic compounds, and the differences with the soil were at least one order.

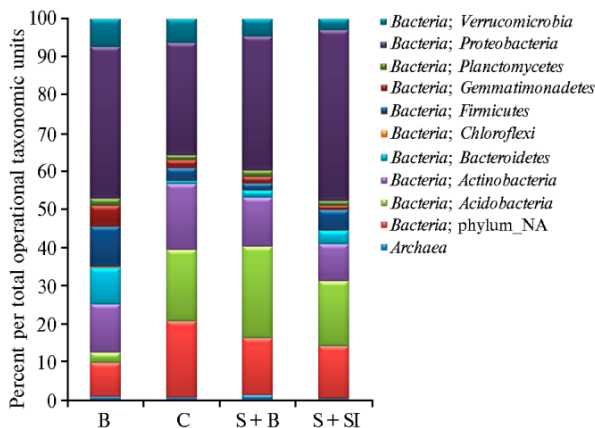


Fig. 1. The composition of the bacterial microbial community of the Barkon biopreparation and sod-podzolic soil when introducing straw and using the Barkon in experiment 1: B — Barkon, C — control (soil), S + B — straw + Barkon, S + SI — straw + soil inoculum.

When analyzing bacterial community of sod-podzolic soils, it was found that microbiomes in three experiments differed significantly between themselves (see Table 3). All three soils formed separate clusters, that is, the soil factor

had the greatest influence on the structure of the microbial community (see Table 3). This conclusion differs from the statement that the main factors in the formation of the microbial community decomposing the straw are climatic conditions, not the type of soil [47]. However, the introduction of straw, the use of biopreparation, the period of analysis and the depth of embedding also had a significant impact on the microbial community composition. For example, when using Barkon as compared to soil inoculum (experiment 1), the number of *Firmicutes* (difference significance is 2.58×10^{-12}) [41], *Bacteroidetes* (6.34×10^{-5}), *Proteobacteria* (1.26×10^{-12}) decreased and the proportion of *Acidobacteria* (8.07×10^{-11}), *Actinobacteria* (1.2×10^{-4}) and archaea (7.8×10^{-4}) increased.

When analyzing the data obtained, particular attention was paid to several of the most important taxa, since a significant part of the microorganisms did not directly participate in the decomposition of straw. It should be noted that each soil was characterized by its own, not always the same set of families in each taxon. Thus, a sensitive indicator of soil pH may be not only the representation of the *Acidobacteria* but also its composition [48]. The experiments established that with a similar content of C_{org} in more acidic soils, *Acidobacteria* of the 1st and 2nd groups prevailed in this taxon (60 and 18%, respectively), in soils with pH close to neutral the 6th group prevailed (30%, for the experiment 1 1–3%), and the 1st and 2nd groups were not identified. *Acidobacteria* of the 6th group responded positively to the introduction of straw on acidic soil (an increase from 1 to 3%).

Actinomycetes play a significant role in the mineralization of hardly decomposable substrates, including straw [2, 17, 49]. The indicating group for the

introduction of straw for sod-podzolic soil in all experiments was the *Micrococcaceae* family. Its representatives are part of the Barkon biopreparation. The significant increase in the proportion of *Micrococcaceae* from all *Actinobacteria* in the variants with straw (Fig. 2) observed in experiment 3 on day 3 remained on day 17, although it was lower, by 10, 17 and 26%, respectively, for control, straw and straw + Barkon. The increased proportion of bacteria of this family was also observed in experiment 1 on day 61 (see Fig. 2).

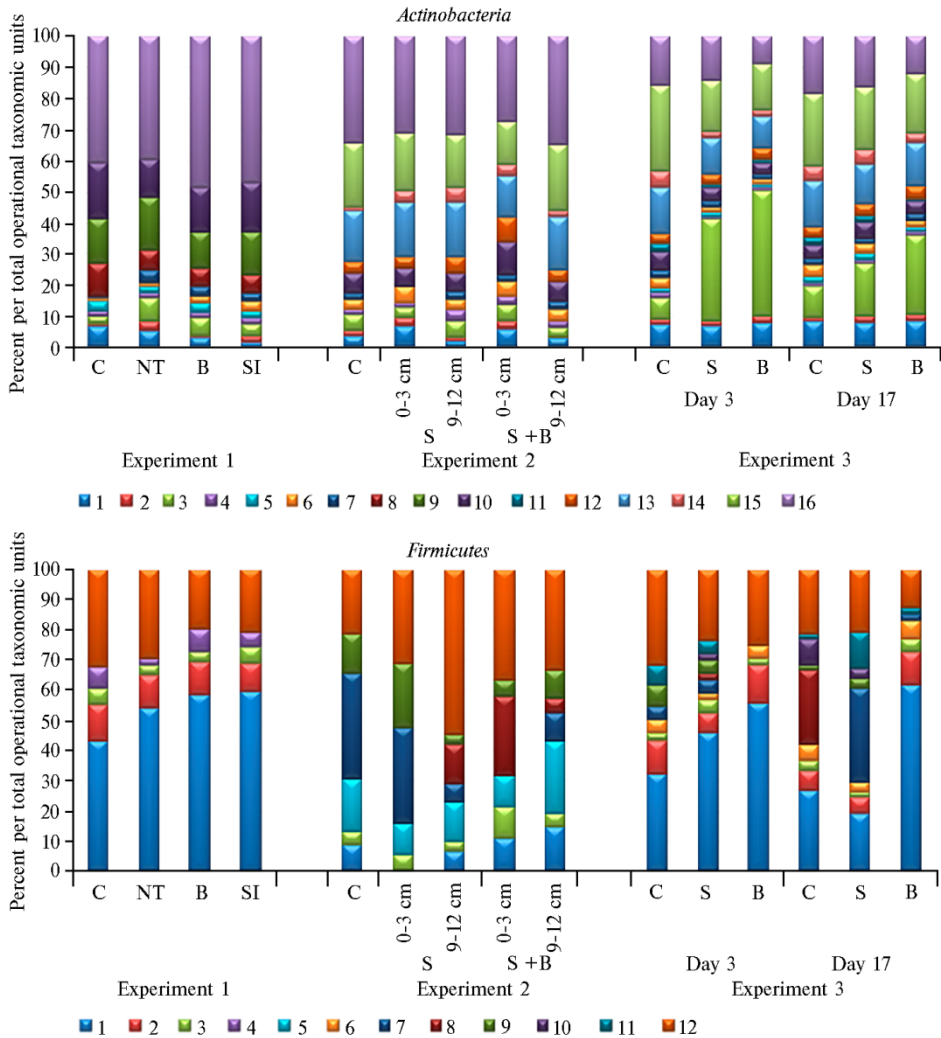


Fig. 2. Composition of individual phyla at the family level in the experiments (share of the total number of operating taxonomic units — OTU) **when introducing straw into the sod-podzolic soil and using the Barkon biopreparation:** C — control, WT — without treatment, B — Barkon, S — straw, SI — soil inoculum. Семейства *Actinobacteria* families: 1 — *Intrasporangiaceae*, 2 — *Microbacteriaceae*, 3 — *Micrococcaceae*, 4 — *Micromonosporaceae*, 5 — *Mycobacteriaceae*, 6 — *Pseudonocardiaceae*, 7 — *Streptomycetaceae*, 8 — *Thermomonosporaceae*, 9 — *Conexibacteraceae*, 10 — *Solirubrobacteraceae*, 11 — *Geodermatophilaceae*, 12 — *Nocardiodiaceae*, 13 — *Gaiellaceae*, 14 — *Patulibacteraceae*, 15 — *Solirubrobacterales*, 16 — прочие. Семейства *Firmicutes*: 1 — *Bacillaceae*, 2 — *Paenibacillaceae*, 3 — *Clostridiaceae*, 4 — *Planococcaceae*, 5 — *Carnobacteriaceae*, 6 — *Alicyclobacillaceae*, 7 — *Staphylococcaceae*, 8 — *Lactobacillaceae*, 9 — *Streptococcaceae*, 10 — *Veillonellaceae*, 11 — [*Tissierellaceae*], 12 — others. For a description of the experiments, see the *Techniques* section.

Spore-forming microorganisms from phyla *Firmicutes* may also be involved in the decomposition of fresh organic matter. The biopreparation had a significant effect on its composition, reducing the number of genera (see Fig. 2).

The greatest influence was observed in the first days after the introducing the straw and the biopreparation (experiment 3), but it remained for later periods: in experiment 1, the number of genera in the control and in the variant with straw with soil inoculum was 17, in the variant with Barkon it was 10. In experiment 2 with soil rich in organic matter, the effect of Barkon occurred only when the preparation was embedded in the lower layer: the number of genera in this phylum for straw and straw with Barkon was 15 and 11, respectively.

The ratio of gram-positive and gram-negative bacteria characterizes the oligotrophic nature of soil processes. It is known that gram-negative bacteria require a richer substrate (fresh organic matter). The decomposition of straw increases their number, especially *Alphaproteobacteria* and *Gammaproteobacteria* [46, 48]. In our experiments, except the experiment 1, there was no increase in gram-negative bacteria, depending on the introduction of straw. Even in experiment 3, when introducing readily degradable oat straw, no increase in the proportion of gram-negative bacteria was found. The reason may be either in differences with the methods used by other researchers (the fatty acid method) or in a high proportion of uncultivated microorganisms.

To assess the influence of the studied factors on the diversity of microbial communities, we used the Shannon index. In experiment 2, when introducing the straw, it was increasing compared to the control as expected. Barkon somewhat reduced the diversity of the microbial community of the soil during the decomposition of straw in the lower layer (control — 5.04; straw in the layer 0-3 and 9-12 cm — 5.47 and 5.57, respectively; Barkon — 6.22 and 5.17). In experiments 1 and 3, no significant differences according to the Shannon index were between the variants. The Sørensen-Czekanowski coefficient of similarity showed that the taxonomic composition of the microbial community of the biopreparation (for experiment 1) differed significantly from all soil variants (0.54-0.59 vs. 0.72-0.75). For experiment 2, the composition of the microbial community decomposing straw in the upper layer had the greatest similarity to the control (0.82), whereas Barkon treatment and decomposition in the lower layer reduced the similarity coefficient to 0.67-0.70. Consequently, when treating with biopreparation, the greatest differences in the composition of microbial communities according to both coefficients were when the straw is incorporated in the lower layer.

The principal component analysis revealed a significant differences between the soil and the biopreparation in experiment 1 (Fig. 3, A). Introduction of Barkon led to significant changes in the structure of the microbial community decomposing straw. Differences between replications were comparable to the differences between the variants (see Fig. 3, B), although at a low level (no more than 9%), especially for the version with straw on day 17. This is probably due to the presence of the detritosphere around the pieces of decomposing straw [46, 50, 51]. Nevertheless, the influence of Barkon on day 3 was quite clear. For experiment 2, a significant difference in microbial communities in different layers is shown (see Fig. 3, B).

The fractal analysis of molecular genetic data presented in the form of a fractal portrait [23, 37, 38] was limited to counting the number of primary fractal groups (PFGs). When searching for PFGs in the portrait, the arrangement of fractal triplets is sequentially analyzed, which include points that differ in the whole number parts of the frequency logarithms and are located on the same line. The absolutely accurate location of three points on one straight line is an exceptional situation. Therefore, all the found PFGs are characterized not only by the taxonomic parameters of the OTU groups, but also by the error (h) of the location of three points on one straight line.

One can assume that the microbial community in which a larger number of PFGs are present has greater consistency and efficiency in the joint transformational activity of microorganisms.

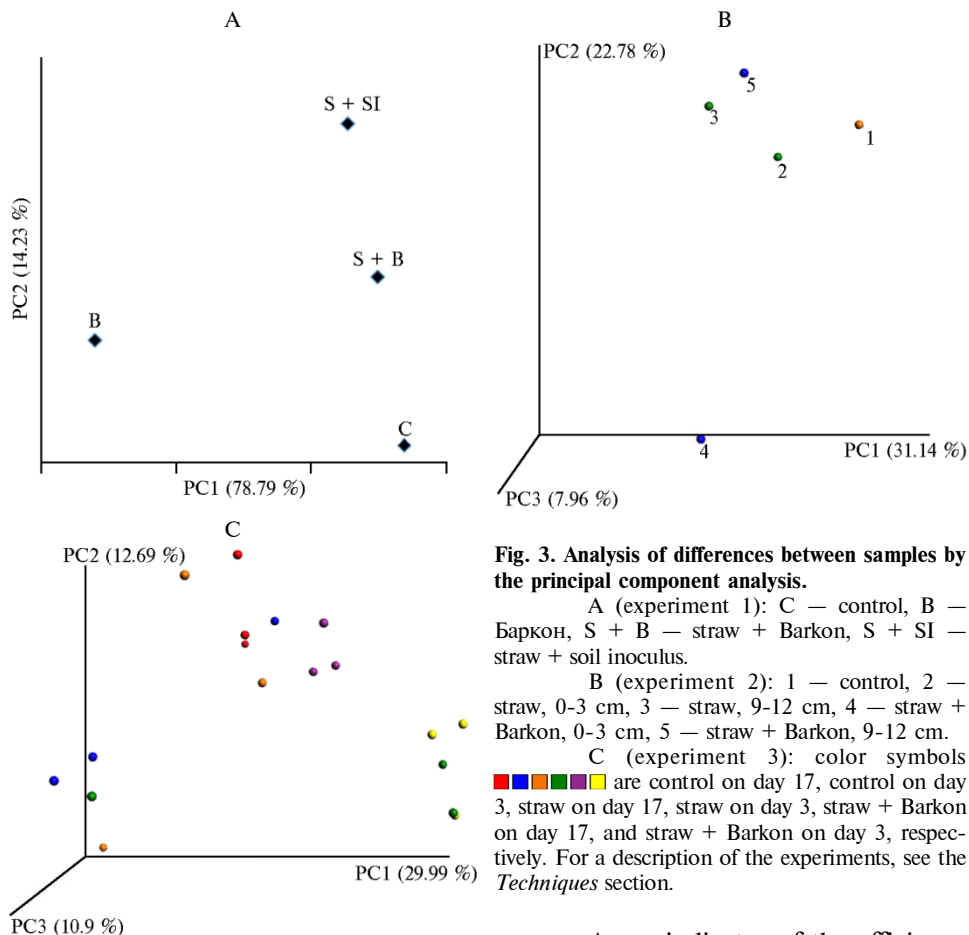


Fig. 3. Analysis of differences between samples by the principal component analysis.

A (experiment 1): C – control, B – Баркон, S + B – straw + Barkon, S + SI – straw + soil inoculus.

B (experiment 2): 1 – control, 2 – straw, 0-3 cm, 3 – straw, 9-12 cm, 4 – straw + Barkon, 0-3 cm, 5 – straw + Barkon, 9-12 cm.

C (experiment 3): color symbols ■ ■ ■ ■ ■ are control on day 17, control on day 3, straw on day 17, straw + Barkon on day 3, straw + Barkon on day 17, and straw + Barkon on day 3, respectively. For a description of the experiments, see the *Techniques* section.

As an indicator of the efficiency of joint transformation activity of the microorganism community, it is proposed

to calculate the index of fractal structures of the microbial community using the following formula:

$$I_F = N_{\Pi\Phi\Gamma}(h) / N_M,$$

where $N_{\text{PFG}}(h)$ is the number of different OTU groups (operational taxonomic unit) in the primary fractal groups identified in the fractal portrait of the microbial community for $\ln(h) \leq -5 \dots -3$; N_M is the total number of OTU groups combining individual OTU with similar frequencies.

For successful utilization of straw, microorganisms form biosystems with the fractal organization of network structures in which the transformational biochemical roles of microorganisms are distributed and the order of joint transformational actions is coordinated. In the case of unsuccessful assembly of a destructive biosystem (for example, in the absence of necessary microorganisms), the efficiency of transformations of plant substrates in the soil is low, which leads to a decrease in the index of fractal structures of the microbial community. For example, on day 17 the index of fractal structures in experiment 3 was lower for non-inoculated straw ($I_F = 0.80$) than for straw and Barkon ($I_F = 0.83$) (Table 3). This means that microorganisms of the biopreparation are embedded in the soil

destructive biosystems in the absence of the necessary microorganisms in it.

For the formation of destructive biosystems, microorganisms need some time to tune in to work together, distribute transformational roles and establish the order of transformational actions. Therefore, the soil microbial community (with straw and Barkon) was not fully tuned ($I_F = 0.79$) in experiment 3 on day 3, but tuned in on day 17 ($I_F = 0.83$). If comparing the variants of experiment 2, which differ in the depth of introducing the straw inoculated by Barkon, then the efficiency of the destructive microbial biosystem was greater when straw was introduced into the upper (see Table 3; $I_F = 0.68$) than in the lower soil layers ($I_F = 0.58$). A likely explanation is the fact that the use of Barkon allows for the best results in terms of the formation of destructive microbial biosystems if the biosystems are characterized by the predominance of aerobic microorganisms, which can receive enough energy for the biochemical transformations of plant substrates by oxidizing organic substances.

In experiment 1, the indexes of fractal structures (see Table 3) in the control and at the straw inoculation with soil extraction were lower ($I_F = 0.44$ and 0.52) than when using straw inoculated with Barkon ($I_F = 0.75$). That is, during production of this biopreparation, microorganisms formed network structures that are ready for joint transformational biochemical activity.

Thus, treatment with the Barkon biopreparation promotes the formation of microbial destructive communities with the highest efficiency of straw transformation into labile organic compounds, and then into soil humus substances. At the decomposition of straw in the soil, as compared to unembedded straw, there is some weakening of the effect of Barkon on the formation of the humification trophic chain, as evidenced by the lack of growth of labile humic substances in the respective variants. The greatest influence on the composition of microbial communities, leading to the decomposition of straw, is exerted by the type of soil, the least — by its chemical, physical, biochemical characteristics. The interrelationship of the soil-microbiological parameters (the number and biomass of microorganisms, respiration) with the taxonomic composition of the microbiocenosis confirms the high response of the composition of the microbial community to various effects while maintaining the core component of the microbiome characteristic of a particular soil. According to the microbial profiles of soil samples, it was for the first time shown that under straw inoculation with the Barkon biopreparation the proportion of the *Micrococcaceae* family increases (*Micrococcaceae* representatives are among the constituent microbial components of this biopreparation). It is established that the minor groups of microorganisms participate equally with the major groups in the formation of network fractal structures. It is shown that the microbiological preparation increases the index of fractal structures of the microbial community, especially in the upper parts of the arable layer, that is, it creates conditions for the effective utilization of straw in the soil, increasing the rate of straw processing into humus compounds.

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