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SOIL BIOLOGY

Dominant Bacterial Taxa in Chernozems and Factors Affecting Their Abundance in the Bacterial Community

K. S. Boyarshin[a, *], V. V. Adamova[a], W. Zheng[a], E. V. Nikitinskaya[b], O. Yu. Obukhova[a], M. V. Kolkova[a], V. A. Nesterenko[c], O. S. Bespalova[a], V. V. Klyueva[a], K. A. Degtyareva[a], L. V. Nesteruk[a], Yu. N. Kurkina[a], O. A. Makanina[a], E. S. Ivanova[b], Zh. Li[d], and I. V. Batlutskaya[a]

[a] *International Laboratory for Applied Biotechnology, Institute of Pharmacy, Chemistry and Biology, Belgorod State University, Belgorod, 308015 Russia*

[b] *Ecological Analytical Laboratory, Department of Biology, Cherepovets State University, Cherepovets, 162600 Russia*

[c] *Dokuchaev Soil Science Institute, Moscow, 119017 Russia*

[d] *Shandong Provincial Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou, 253023 China*

**e-mail: kboyarshin@mail.ru*

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Abstract—Families and genera of bacteria that predominate in chernozems of the forest-steppe zone have been identified. Microbiological profiling of samples of plowed and unplowed chernozems is performed, using the 16S rRNA gene sequencing, at different phases of the growing period: in June and August. Changes in the proportion of individual bacterial families, depending on land use and time, are shown. Correlations between the occurrence of bacterial families and chemical parameters of soil have been revealed. The predominant role of nitrates in the formation of the community structure and the important contribution of the organic carbon content, soil moisture, and pH in this process are shown. Despite the revealed differences in the proportions of the studied families, depending on land use and the sampling period, the set of dominant bacterial families in the studied samples remains stable. The first six dominant families comprise about 1/4 of the entire community, and the first 20 ones make up about 40%. The obtained results create prerequisites for further study of the variability of the taxonomic composition of the bacterial community in chernozems under various biotic and agrochemical conditions.

Keywords: soil microbiota, microbiological profiling, 16S rRNA, chernozem, plowed lands

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INTRODUCTION

Chernozems [3] occupy significant areas on the Eurasian continent [24] and are intensively used in agriculture [11, 13]. This is related to their properties such as high organic matter content [25] and pH close to neutral [36]. The great amount of organic matter in soil provides the retention of moisture and nutrients [39, 41], which increases natural soil fertility and the efficiency of applied fertilizers. These properties of chernozems determine their high value and their specific role as a reference plowed soil. A large number of works tackle their physicochemical characteristics [42], biological diversity, and fertility maintenance [40].

The aim of this work is to identify bacterial taxa at the level of family and genus, predominating in chernozems of the forest-steppe zone, by the example of Belgorod oblast, and to assess the effect of various agrochemical factors on them. There is a tendency in modern soil microbiology to the wide use of statistical methods, which enable soil scientists to characterize and compare soil microbial communities in general [8, 28, 43]. However, there is no aim to analyze in detail the occurrence of individual families and genera of microorganisms. This is related to methodological problems, because the reliability of quantitative data on individual families and genera may be questioned, because PCR primers are mainly bound to sequences, characterizing particular taxa [21, 34].

We assume in this work that PCR biases would not distort the taxonomic structure of a community beyond recognition, if a sufficient number of families and genera, composing significant proportions in the community, are included in it. Not only the mean occurrence of taxa are taken into account, but also its variance, indicative of their status:, which microorganisms are less typical, but also probable in nature.

We analyzed the most numerous 20 families, which on average occupy about 40% in the community in total and obviously play significant roles in its biochemical activity. The compilation of such a list of groups, dominating the bacterial microbiota in chernozems, is of fundamental interest for further study

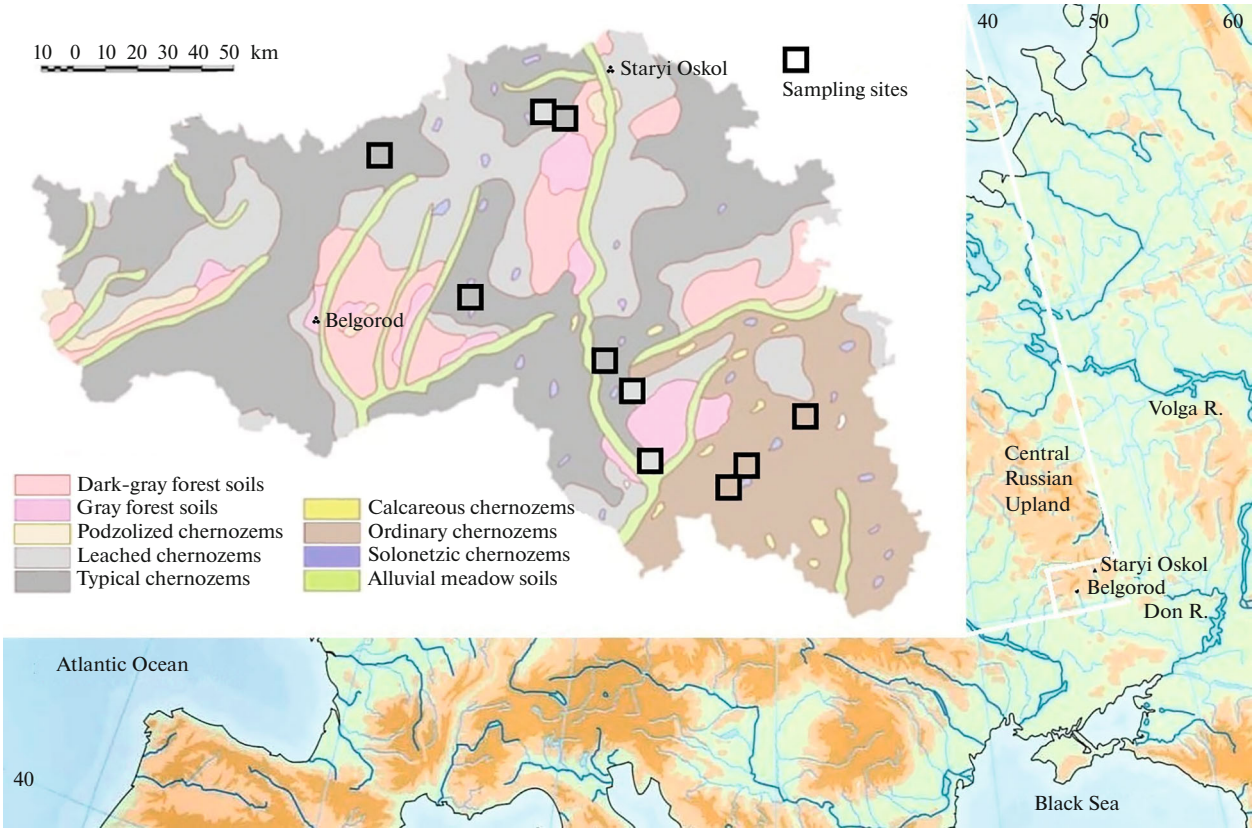

Fig. 1. Sampling sites on the soil map of Belgorod oblast of Russia.

concerning the distribution of the main biochemical functions in communities, such as chemical transformation of nutrients used by plants, conservation or consumption of soil organic matter, and the effect on pH. We have also revealed correlative dependences of the shares of bacterial families in the community on the land use and agrochemical parameters.

OBJECTS AND METHODS

Soil sampling. The study was performed in Belgorod oblast of Russia, in the south of the Central Russian Upland, in the basins of the Oskol and the Severskii Donets rivers, which are components of the Don River system (Fig. 1). Belgorod oblast is located in the forest-steppe zone on an elevated plain with pronounced erosional landforms, and the altitude above sea level averages 200 m. The mean annual air temperature at different sites of the region varies within 5.4–6.8°C. January is the coldest month, the frostless period lasts 155–160 days, and the duration of sunshine is 1800 h/yr. Soil freezes in winter to a depth of 0.5–1.0 m. The annual precipitation varies from 540–550 to 400 mm in various parts of the region. The area under forest comprises 9.8% of the oblast area [1]. Zonal soils are represented by various subtypes of chernozems, occupying 77% of the area, and by forest soils (15% of the area) [2].

Typical chernozem (Haplic Chernozem) is the most widespread soil in the study area. Its vast areas are adjacent to those of leached chernozems (Luvic Chernozems), which also occupy large areas. The southeastern part of the region is dominated by ordinary chernozems (Haplic Chernozems), which is typical for more dry conditions. In addition, there are less common varieties of chernozems in the region, allocated to particular elements of topography. These are alluvial-meadow chernozems (Gleyic Chernozems (Fluvic)) common in river floodplains. Erosion processes are responsible for outcrops of chalk rocks, and calcareous chernozems (Calcaric Phaeozems) are often formed on the slopes. There are also solonetzic chernozems (Gleyic Chernozems (Protosodic)).

We studied typical, leached, and ordinary chernozems and took 40 soil samples (Table 1). The sampling sites were allocated to the areas, where plowed fields were adjacent to unplowed ones (cultivated and noncultivated soils). The plowed areas of all studied chernozems were sown by winter wheat, and the vegetation of the unplowed areas included forb communities or deciduous trees with grass layer. Each sample consisted of 20 parts sampled in the humus-accumulative horizon (A1) from a depth of 5–15 cm at a distance of 3–4 m from each other. The sampling sites of plowed and unplowed soils of each variety of chernozem were located at a distance of 10–20 m from each other. The

Table 1. Number of samples of plowed (p) and unplowed (up) chernozems of different subtypes (the A1 horizon from 5- to 15-cm deep)

Period	Chernozem						Total
	typical		leached		ordinary		
	p	up	p	up	p	up	
End of June 2022	4	4	3	3	3	3	20
End of August 2022	4	4	3	3	3	3	20
Total	8	8	6	6	6	6	40

mass of each sample was about 1 kg. The samples were taken at the same sites twice: at the end of June and at the end of August, 2022.

Microbiological profiling. The experiments were performed according to [9]. On the day of sampling, each soil sample was thoroughly ground to a particle size of less than 2 mm and mixed. Plant roots and other identified organic residues were removed. After mixing, the samples were frozen without pre-drying and stored at a temperature of –80°C. DNA was isolated from a 0.3 g sample, using a FastDNA Spin Kit for Soil DNA Extraction (MP Biomedicals, United States). Two-stage PCR was performed to obtain amplicons of a fragment of the 16S rRNA gene, containing hypervariable regions V3 and V4. After purification on Agencourt AMPure XP magnetic particles (Beckman Coulter, Unites States), amplicons were used to prepare libraries and for paired-end sequencing on an Illumina MiSeq sequencer (Illumina, United States). At least 10000 paired readings were obtained for each sample. The QIIME 1.9.1 software package was used for primary data processing [10], in particular, sequences with a quality index less than Q30 were excluded. The taxonomic identification of the obtained sequences was performed by the Silva database [27] version 132. The classification threshold of 97% was applied. The occurrence of various taxa was calculated by algorithms elaborated for this purpose in the Python 3 programming language.

Agrochemical analysis. Soil pH was determined in a solution of 1 M KCl at the soil to solution ratio of 1 : 2.5, using a glass electrode (*GOST* 26483-85). The mass fraction of organic matter (C_{org}, %) was detected by the photometric method by Tyurin based on oxidation with a solution of $K_2Cr_2O_7$ in sulfuric acid followed by determination of the content of trivalent chromium at an absorbed wavelength of 590 nm (*GOST* 26213-2021). Mobile species of P and K (recalculated per P_2O_5 and K_2O, mg/kg of soil, respectively) were determined by the Chirikov method based on the extraction of mobile phosphorus and potassium compounds from soil with 0.5 M acetic acid solution (the soil to solution ratio of 1 : 25) with further detection of phosphorus as blue phosphorus-molybdenum complex on a photoelectrocolorimeter and of potassium on a flame photometer (*GOST* 26204-91). The content of nitrates ($N-NO_3$, mg/kg of soil) was measured after their extraction from soil with 1 M K_2SO_4 solution at the ratio of the soil sample mass to the solution volume of 1 : 2.5. Nitrates were determined in the extract by an ion-selective electrode (*GOST* 26951-86). The ammonium content ($N-NH_4$, mg/kg of soil) was determined by extracting it from soil with 1 M KCl solution and obtaining a colored indophenol compound by exposure to sodium hypochlorite and salicylate in an alkaline medium with further photometry of the colored solution (*GOST* 26489-85).

Statistical data processing. The Permutational Multivariate Analysis of Variation (Permanova) with distance matrices [29] was used to analyze the effect of soil subtype, land use, and soil chemical properties on the structure of the microbial community [7]. The interaction of independent variables with each other was not taken into account, and the taxonomic structure of the community at the family level was taken as the dependent variable. The dissimilarity indices were calculated by the Bray-Curtis method, and a matrix of distances between communities was compiled on their basis and used for the multidimensional analysis of variance.

We tested differences in the proportions of microorganisms of each family in microbial communities of chernozems, differing in: (1) land use type, (2) chernozem subtype, and (3) time of soil sampling, using the method of nonparametric Kruskal–Wallis analysis of variance [23]. To analyze the differences in the occurrence of families in various subtypes of chernozem, pairwise multiple comparison by the Wilcoxon rank sum test [44] with correction of *p*-values by the Beniamini–Hochberg method was used.

The correlation analysis of the relationship between the proportion of a family in the community and the chemical parameters of soil was performed, using the Spearman correlation coefficient. All statistical calculations were performed in the R environment version 4.1.2, using the Vegan package [14].

RESULTS AND DISCUSSION

Bacterial families, dominating the microbiota of chernozem. The analysis of bacterial communities in adjacent plowed and unplowed areas at the end of June and end of August shows that the mean proportions of

Table 2. The proportion of the most numerous families in plowed (p) and unplowed (up) chernozems at different phases of the growing season (June and August). Statistically significant domination in soils is given in bold and the same for one of the months, %, is underlined; mean ± standard deviation

Family	June		August		Mean ($n = 40$)
	p ($n = 10$)	up ($n = 10$)	p ($n = 10$)	up ($n = 10$)	
Gemmatimonadaceae	**7.59** ± 1.66	3.87 ± 1.99	**6.85** ± 1.25	3.32 ± 1.87	5.41 ± 2.49
Chitinophagaceae	4.54 ± 0.50	4.53 ± 1.06	5.70 ± 0.79	4.70 ± 1.05	4.87 ± 0.98
Sphingomonadaceae	6.31 ± 2.25	6.38 ± 2.81	**4.29** ± 1.66	1.33 ± 0.77	4.58 ± 2.84
Xanthobacteraceae	3.03 ± 0.60	4.56 ± 1.94	2.79 ± 0.65	**4.86** ± 1.08	3.81 ± 1.47
Chthoniobacteraceae	2.10 ± 1.05	2.52 ± 1.97	2.50 ± 0.98	**4.29** ± 2.61	2.85 ± 1.92
Rubrobacteriaceae	2.51 ± 0.87	3.01 ± 1.23	1.73 ± 0.60	2.30 ± 0.93	2.39 ± 1.01
Burkholderiaceae	2.30 ± 1.33	2.18 ± 0.76	**1.65** ± 0.40	1.10 ± 0.39	1.81 ± 0.92
Microscillaceae	0.88 ± 0.75	**2.12** ± 0.72	1.64 ± 0.95	2.00 ± 0.99	1.66 ± 0.96
Nocardioidaceae	1.36 ± 0.27	**2.03** ± 0.48	1.37 ± 0.40	1.37 ± 0.15	1.53 ± 0.44
Micromonosporaceae	0.97 ± 0.31	1.40 ± 0.70	1.42 ± 0.53	1.80 ± 0.66	1.40 ± 0.62
Solirubrobacteraceae	1.18 ± 0.32	**1.97** ± 0.59	0.92 ± 0.25	**1.21** ± 0.17	1.32 ± 0.53
Pedosphaeraceae	1.07 ± 0.49	0.79 ± 0.47	1.59 ± 0.38	1.73 ± 0.46	1.29 ± 0.58
Pyrinomonadaceae	**1.05** ± 0.37	0.53 ± 0.23	1.62 ± 0.69	1.93 ± 0.67	1.28 ± 0.74
SC-I-84 (Burkholderiales)	**1.90** ± 1.02	0.97 ± 0.49	1.34 ± 0.77	0.73 ± 0.52	1.23 ± 0.83
Ilumatobacteraceae	0.86 ± 0.26	**1.72** ± 0.91	1.07 ± 0.28	1.24 ± 0.47	1.22 ± 0.62
Nitrosomonadaceae	**1.33** ± 0.45	0.91 ± 0.25	1.35 ± 0.38	1.20 ± 0.41	1.20 ± 0.41
Xanthomonadaceae	1.48 ± 1.13	1.15 ± 0.58	**1.42** ± 0.64	0.52 ± 0.11	1.14 ± 0.78
Blastocatellaceae	1.06 ± 0.37	0.99 ± 0.31	**1.46** ± 0.48	0.67 ± 0.27	1.04 ± 0.45
Haliangiaceae	1.03 ± 0.51	0.76 ± 0.33	1.15 ± 0.37	1.05 ± 0.40	1.00 ± 0.42
Caulobacteraceae	0.91 ± 0.48	1.40 ± 0.62	0.70 ± 0.27	0.54 ± 0.21	0.89 ± 0.52
Total	43.43	43.77	42.56	37.86	41.91

the dominant families are similar (Table 2). The proportions of all the considered families may repeatedly differ, depending on the location (Table 3). These families may be characterized as permanent and significant participants of microbiota functioning in chernozems. The range of their variation may be a consequence of a particular ecological substitution, at which ecological niches of the representatives of different families are considerably overlapped [16, 20]. This conclusion is confirmed by the relatively constant means preserved even under different environmental conditions, such as agricultural activity or its absence, as well as at different stages of the growing season. This stability potentially enables a more detailed study of the distribution of basic ecological functions in microbial communities of chernozems.

Microbiological profiling shows that there are bacteria assigned to at least 237 families in the samples. The first six ones presented in the tables on average comprise about a quarter of the total composition of the bacterial community, taking into account unidentified sequences. The first twenty families form about 40%. Thus, the list of families considered in this work characterizes a significant part of the microbiota.

Most families include particular dominant genera. Their proportions in the community vary even more widely than those of families, which indicates even greater ecological substitution at this taxonomic level. The high mean proportion of some bacterial genera should obviously be considered as an evidence of their participation in performing the most important biochemical functions of bacteria in soil. Thus, a comprehensive study of biochemical capabilities of their representatives may help to assess the development of particular processes and their role for the soil ecosystem.

Unfortunately, there are few works that enable us to compare the results obtained. Studies, using microbiological profiling, are usually aimed at comprehensive characteristic of microbial communities: of their biodiversity, the stability of their structure, and the effect of particular factors, without identification of dominant taxa at the level of families and genera. Nevertheless, there are works, containing such information.

Table 3. Dominant genera of the 20 largest families of soil bacteria. Ranges of mean percentages in microbial communities of chernozems for families and genera are given. Genera that may comprise more than 1% of the entire community are shown; if no such genera are identified in the family, the most common genus is given and underlined. The data in column *Mean* are given for genera, and the mean shares of families are given in Table 2

Family	Proportion, %	Genus	Proportion, %	Mean, %
Gemmatimonadaceae	1.62–10.19	*Gemmatimonas*	0.2–5.8	2.15 ± 1.4
Chitinophagaceae	3.11–6.71	*Ferruginibacter*	0.17–1.67	0.55 ± 0.32
		Flavisolibacter	0.02–1.72	0.66 ± 0.39
		Terrimonas	0.1–1.64	0.54 ± 0.26
Sphingomonadaceae	0.42–11.35	*Sphingomonas*	0.18– 8.84	3.58 ± 2.42
		Novosphingobium	0.0–1.13	0.20 ± 0.20
		Altererythrobacter	0.0–1.01	0.29 ± 0.24
Xanthobacteraceae	1.5–8.85	*Bradyrhizobium*	0.6–3.51	1.53 ± 0.63
Chthoniobacteraceae	0.58–10.52	*Cand. Udaeobacter*	0.25–9.64	2.21 ± 1.81
		Chthoniobacter	0.2– 1.32	0.64 ± 0.28
Rubrobacteriaceae	0.75–4.96	*Rubrobacter*	0.75–4.96	2.39 ± 1.01
Burkholderiaceae	0.52–5.34	*Massilia*	0.0–2.05	0.30 ± 0.42
Microscillaceae	0.16–3.49	Chryseolinea	*0.0–0.88*	*0.18 ± 0.22*
Nocardioidaceae	0.9–3.18	*Nocardioides*	0.37–1.84	0.86 ± 0.37
		Kribbella	0.19–1.12	0.44 ± 0.17
Micromonosporaceae	0.48–2.95	*Actinoplanes*	0.0–1.53	0.29 ± 0.30
Solirubrobacteraceae	0.62–2.79	*Solirubrobacter*	0.31–2.15	0.86 ± 0.41
Pedosphaeraceae	0.18–2.48	ADurb.Bin063-1	*0.0–0.37*	*0.09 ± 0.10*
Pyrinomonadaceae	0.17–2.92	RB41	0.17–2.92	1.28 ± 0.74
SC-I-84 (Burkholderiales)	0.08–3.18	–	–	–
Ilumatobacteraceae	0.33–3.6	*Ilumatobacter*	0.0–1.48	0.30 ± 0.26
Nitrosomonadaceae	0.57–1.99	*Ellin6067*	0.14–1.18	0.60 ± 0.24
		MND1	0.06–1.06	0.47 ± 0.28
Xanthomonadaceae	0.31–4.46	*Stenotrophomonas*	0.0–1.95	0.11 ± 0.30
		Lysobacter	0.04–1.66	0.53 ± 0.33
		Pseudoxanthomonas	0.0–1.07	0.14 ± 0.21
Blastocatellaceae	0.31–2.17	Aridibacter	*0.0–0.28*	*0.01 ± 0.04*
Haliangiaceae	0.31–2.16	*Haliangium*	0.31–2.16	1.00 ± 0.42
Caulobacteraceae	0.23–2.13	*Phenylobacterium*	0.02–1.2	0.36 ± 0.22

It is shown [18] that the most common families in typical chernozem (plowed and unplowed areas, Kursk oblast, about 100 km to the northwest of the area of this study) are represented by Bradyrhizobiaceae, Mycobacteriaceae, Streptomycetaceae, Pseudomonadaceae, Planctomycetaceae, Micromonosporaceae, Xanthomonadaceae, Sphingomonadaceae, Acidobacteraceae, and Comamonadaceae. In our study, the Micromonosporaceae, Xanthomonadaceae, and Sphingomonadaceae families are also assigned to predominating ones. As compared to our results, the Mycobacteriaceae, Streptomycetaceae, and Pseudomonadaceae families are significantly more widespread according to data in [18]. At the level of genera, the *Bradyrhizobium* genus is very numerous according to our results and those given in [18]. Both studies show that representatives of *Solirubrobacter*, *Sphingomonas,* and *Chtoniobacter* genera are abundant.

The work, reflecting differences in the occurrence of bacterial taxa in plowed and unplowed typical chernozem, has been performed in Kursk oblast [4]. It is shown that the abundance of *Pseudomonas* (the family Pseudomonadaceae), *Bacillus* (Bacillaceae), *Kaistobacter* (Sphingomonadaceae), and *Candidatus Solibacter* (Solibacteraceae) genera is high. The *Kaistobacter* genus is a heterotypic synonym of *Sphingomonas* [32], the occurrence of which in our study is high.

Samples of plowed and unplowed typical chernozem in [33] are taken in Voronezh oblast. Data on the dominant families and genera show the abundance of

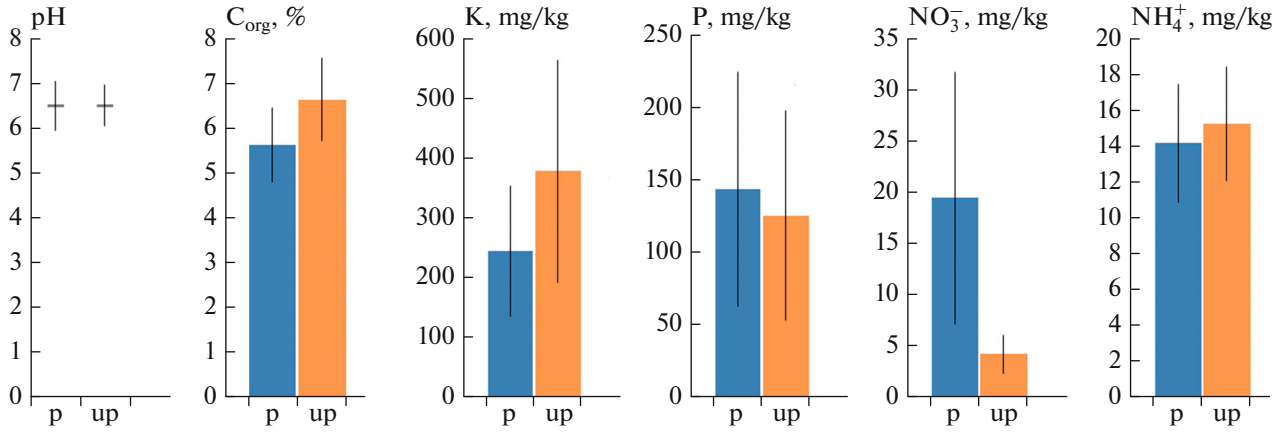

Fig. 2. Chemical characteristics of plowed (p) and unplowed (up) chernozems with their standard deviations: pH KCl, mass fraction of organic substances (C_{org}, %), mass concentrations of exchangeable potassium recalculated per K_2O, exchangeable phosphorus recalculated per P_2O_5, and NO_3^- and NH_4^+ recalculated per nitrogen.

representatives of the Chitinophagaceae families, which coincides with our results, as well as a greater number of representatives of the Gaiellaceae, Hyphomicrobiaceae, and Syntrophobacteraceae families. The abundance of *Chthoniobacter* genus is high, and the *Rhodoplanes* genus is still higher, while it averages only 0.34% in our samples.

The differences in the list of dominant taxa in chernozems of the same subtype in neighboring Belgorod, Kursk, and Voronezh oblasts may be explained by various reasons, including methodological ones. The taxonomic nomenclature used in articles published in various years differs, which makes difficult the comparison of published data. There may be errors of applied methods of microbiological profiling, which underestimate or overestimate the proportions of particular taxa. The main influence is exerted by different efficiency of amplification of hypervariable sections of 16S rRNA genes with various nucleotide sequences, using particular pairs of primers. The microbiological profiling in this work is performed, using hypervariable regions V3 and V4 of 16S rRNA gene [9]. Region V4 is used in [4, 33], and work [18] is based on the metagenomic shotgun sequencing. Finally, the ecological substitution of species assigned to different taxa based on the coincidence of the available ecological niches may result in significant differences in the taxonomic structure of communities even under similar soil conditions. It should be taken into account that chemical conditions in the same region and the same soil type may differ for casual causes or due to the use of different farming methods. These factors, related to the PCR error, in particular, make especially useful the comparison of data of various studies and generalization of the information obtained. They make necessary the use of the same methods and sampling zones in experimental studies with molecular profiling of microbial communities, if they are aimed at the study the ecological properties of species or groups of microorganisms.

Agrochemical analysis of plowed and unplowed chernozems. The main chemical parameters of the studied plowed and unplowed soils are similar (Fig. 2). The mean pH values are practically equal despite the fact that plowed soils tend to acidification [37]. This is explained by the efficiency of lime application widely used in the region. Slightly acid and neutral reaction is typical for chernozems [5] with a tendency to higher acidity in less arid parts of their area. The pH range is 5.8–7.3 for plowed soils and 5.9–7.2 for unplowed ones.

The proportion of organic compounds in plowed soils is on average slightly lower as compared to unplowed soils. This parameter is in general high, which is typical for chernozems [19]. It ranges within 3.9–6.6% for plowed soils and within 5.6–8.6% for unplowed ones. High organic matter content should favor the development and diversity of microbial community [35]. In our study, neither the organic matter content, nor pH, nor other chemical parameters exert a statistically significant effect on the bacterial community in general (Table 4).

The studied sampling is characterized by a wide range of concentrations of available potassium. Its content in plowed soils is on average lower (122–516 mg/kg) as compared to unplowed ones (117–630 mg/kg). The range of available phosphorus is also wide: 60–289 mg/kg for agricultural lands and 44–237 mg/kg for uncultivated soils. As a result of the used agricultural technology, the phosphorus budget is closer to the natural level than the potassium budget.

Plowed soils are characterized by an increased mean content of nitrate nitrogen, and this is the only statistically significant difference in chemical parameters between plowed and unplowed chernozems in the

Table 4. Permutation multidimensional analysis of the effect of factors on the structure of soil bacterial communities. The single factor, which exerts statistically significant impact, is highlighted in bold and means plowing or its absence

Factor	D_f	Σ of squares	R^2	F	$Pr(>F)$
Subtype of chernozem	2	0.04754	0.10129	1.5543	0.170
Land use	1	0.13671	0.29128	8.9397	**0.001**
Moisture content	1	0.01809	0.03855	1.1832	0.302
pH	1	0.01974	0.04206	1.2908	0.258
Organic matter (C_{org})	1	0.02346	0.04999	1.5341	0.177
Potassium	1	0.01235	0.02632	0.8078	0.524
Phosphorus	1	0.01170	0.02493	0.7651	0.566
Nitrate	1	0.02831	0.06032	1.8513	0.132
Ammonium	1	0.01470	0.03132	0.9612	0.410

Table 5. Correlation coefficients between the proportions of dominant families in the soil community and chemical parameters of soil. Only values with a significance level below 0.05 are given

Family	Moisture content	pH	C_{org}	P	NO_3^-
Gemmatimonadaceae	0.47		–0.57		0.82
Chitinophagaceae					
Sphingomonadaceae					0.82
Xanthobacteraceae			0.46		–0.78
Chthoniobacteraceae				–0.47	
Rubrobacteriaceae					
Burkholderiaceae					
Microscillaceae		0.58			
Nocardioidaceae					
Micromonosporaceae					
Solirubrobacteraceae			0.67		
Pedosphaeraceae					
Pyrinomonadaceae					
SC-I-84 (Burkholderiales)	0.47	–0.46			0.44
Ilumatobacteraceae					–0.47
Nitrosomonadaceae			–0.56		
Xanthomonadaceae	0.47				0.76
Blastocatellaceae	0.46				0.72
Haliangiaceae					
Caulobacteraceae					0.48

analyzed sampling. The parameter varies widely: from 8 to 46 mg/kg. In unplowed soils, its variation is also significant: 2–7 mg/kg. The mean content of ammonium nitrogen remains similar in samples of plowed and unplowed soils: 10–20 and 9–21 mg/kg, respectively.

Despite the fact that chemical properties of plowed soils in a particular area are determined by agricultural measures, the ranges of the studied parameters are of great importance for this study, because they provide the diversity of sampling for the analysis of correlation between chemical parameters and the occurrence of representatives of particular bacterial families (Table 5).

Ecological features of individual families, dominating the microbiota of chernozem. The end of June and the end of August represent two different phases of the growing season, differing in the status of the plant cover of both plowed and unplowed soils. The haymaking season is terminated in unplowed areas by the end of June in the studied region. The reproduction of biomass of herbaceous plants is slowed down in the period from the end of June to the end of August, which is related to a decrease in the physiological activity of roots. Thus, the environmental conditions in the subsurface soil layer are significantly changed, which may cause variations in the composition of the soil microflora.

Changes on croplands under winter wheat are even more pronounced in this period. Ears are already formed by the end of June, and the drying of the shoots begins. Since that time, plant growth in such fields practically stops, and dead roots and then plowed straw begin to decompose. Ecological niches of bacteria related to root exudation disappear, and niches associated with the processing of plant residues become larger.

Typical chernozem in work [6] describing the changes in microbial communities composition during the growing season significantly differs in the content of bacterial types in May, July, and September. Unfortunately, these differences are not analyzed for other taxonomic levels. Seasonal changes in the composition of the microbiota for typical chernozem are shown in this work.

The analysis of differences in the proportions of particular dominant bacterial families between plowed and unplowed chernozems in June and August (Table 2) reveals six families, the participation of which significantly changes, depending on the stage of the growing cycle (the data are underlined). For two families—Solirubrobacteraceae and Pedosphaeraceae—the changes for plowed and unplowed soils display the same trend. The differences between plowed and unplowed soils in various months are reliable only for the families Gemmatimonadaceae and Solirubrobacteraceae. The latter reliably prefers June and unplowed soils.

The permutation multidimensional analysis of variance of all the studied factors shows a significant effect of only one factor—land use—on the structure of the bacterial community in chernozem. Agricultural use of soils is associated with specific factors, which may affect their microflora. First, this is the destruction of the natural soil structure and its over-compaction, resulting in poorer aeration and drainage with longer moisture retention in the subsurface layer. The samples of plowed chernozems are characterized by increased mean nitrate content as compared to the samples of unplowed soils. However, the individual impact of these factors on the taxonomic structure of the community is not significant, which may be explained by different sensitivity of various components of the community to their effect. Land use, in turn, is a complex of factors that in total affects a significant part of the community and thus cause its significant restructuring. The factors, appearing during the agricultural use, which have not been considered, should not be excluded.

The correlation analysis has revealed the dependence of the proportion of particular families in the community on chemical characteristics of the soil. The content of nitrates is the factor, most significantly affecting the composition of the community: this effect is positive for six of the dominant families and negative for two of them. The question may arise whether this correlation is not a consequence of the fact that high concentrations of nitrates are related to agricultural use? However, the wide range of concentrations in the sampling of plowed soils testifies to the reliability of the obtained result.

Four families are characterized by a dependence on the content of organic substances: two by positive and two by negative one. The negative correlation is probably explained by a favorable impact of the high content of organic substances on the number of particular competitors or antagonists, affecting a significant part of representatives of these two families. Four families depend on the water content. They all prefer higher moisture within the analyzed range between 17.5 and 33.4%. Soil pH affects three families: two of them prefer more alkaline medium, and one is related to acidic conditions. The proportion of representatives of Chthoniobacteraceae family is characterized by a negative dependence on the content of available phosphorus. The correlation of potassium and ammonium contents with the shares of any common family in the community is absent. The abundance of representatives of the ammonium-oxidizing Nitrosomonadaceae family does not correlate with the content of ammonium or nitrate. This may be related to a rather narrow range of ammonium content in the sampling.

Five families do not depend on any of the considered chemical soil parameters. The Rubrobacteriaceae and Haliangiaceae families are characterized by the absence of dependence on the land use type or on the phase of the growing season. This may be typical for groups of organisms, occupying ecological niches with the slowest biochemical processes in the soil, and thus the least liable to seasonal changes. Ranges of chemical factors in natural and cultivated soils may be too narrow to analyze their impact on organisms with a wide norm of reaction. The accumulation of dead DNA of various bacterial taxa in soils is a specific problem.

The individual analysis of the most common families shows that the proportion of Gemmatimonadaceae family positively correlates with the concentration of nitrate ions and negatively correlates with the content of organic substances. The mean proportion of its representatives is approximately two times higher in plowed soils than in unplowed ones in both June and August samples, and this difference is statistically significant. The proportion of Gemmatimonadaceae family correlates with moisture content. The *Gemmatimonas* genus is the most common among its representatives, and the *Gemmatirosa* genus is also present. Representatives of the Gemmatimonadaceae family are motile rod-shaped non-spore-forming obligate aerobic bacteria that can be cultured in the medium with low concentrations of glucose, peptone, and other organic substances [45].

The occurrence of Sphingomonadaceae family correlates with the concentration of nitrate ions. It is almost equal in plowed and unplowed soils and is higher in June than in August. The family is represented in chernozems by at least 14 genera dominated by *Fer-*

ruginibacter, *Flavisolibacter*, and *Terrimona*. Their common features include aerobic activity and chemoorganotrophy. The *Sphingomonas* genus is capable of producing exopolysaccharides (sphinganes), and some its species can decompose polyaromatic compounds [22]. The occurrence of *Blastomonas* genus, which contains bacteriochlorophyll *a* [17], is low.

The proportion of Xanthobacteraceae family is on the contrary characterized by a negative correlation with the content of nitrates. Statistically significant differences between plowed and unplowed soils are formed by August. There are seven genera in the samples, dominated by the *Bradyrhizobium* genus, and proportions of *Rhodoplanes* and *Pseudolabrys* in the community may be significant (0.04–0.86 and 0.04–0.82%, respectively). The family is characterized by an aerobic chemoheterotrophic type of nutrition, although facultative chemolithoautotrophy with the use of hydrogen or reduced sulfur also occurs. The genera capable to fix atmospheric nitrogen, *Bradyrhizobium* genus in particular, are widespread [26].

The calculations show a negative relationship between the occurrence of the Chthoniobacteraceae family and the content of mobile phosphate. There is statistically a larger proportion of its representatives in unplowed soils in August. Three genera are found in the samples: *Chthoniobacter*, *Candidatus*, *Udaeobacter,* and LD29. The latter not included in Table 3 comprises only to 0.03% of the community. The family includes immobile non-spore-forming aerobic bacteria, and the type species is able to use carbohydrate components of plant biomass and pyruvic acid for growth. Some species are endosymbionts of nematodes and become anaerobic [31].

The proportion of Chitinophagaceae family in plowed and unplowed soils is almost similar in June, but becomes significantly higher under plowed conditions by August. This family includes 17 bacterial genera represented in the samples, in which *Ferruginibacter*, *Flavisolibacter,* and *Terrimonas* predominate. Representatives of the family are aerobes or facultative anaerobes, and species of *Chitinophaga* genus are capable of hydrolysis of cellulose and chitin. Some representatives can move by sliding and form microcysts [30].

The Rubrobacteraceae family is an example of families, which do not depend on any of the factors studied. Its single genus *Rubrobacter* is immobile, does not form spores, and is able to use a large number of monosaccharides without acid formation. Under anaerobic conditions, *R. radiotolerans* reduces nitrates to nitrites [38]. It is able to survive drying and ionizing radiation [15]. *Rubrobacter* is in particular revealed in samples from the surface of limestone masonry of historical buildings [12]. It cannot be excluded that the preservation of dormant and inactivated cells may result in the accumulation of inactive DNA of the Rubrobacteraceae family in soils. However, the data obtained testify to a decrease in the proportion of DNA of this family in the samples by more than 30% within two months. Thus, the possible presence of dead DNA in soils does not prevent in this case the detection of a change in the proportion of the family in the community during the chosen time period.

CONCLUSIONS

The compiled list of the main components of the bacterial microbiota in chernozems at the level of families and genera enables us to identify typical groups of bacteria, high proportions of which testify to their involvement in essential ecological functions provided by microorganisms in the soils. The main conclusions based on the results obtained are the following.

(1) The taxonomic structure of the bacterial community in the forest-steppe chernozems is quite stable despite the differences depending on land use and the stage of the growing season.

(2) The pronounced domination of particular genera in the analyzed families makes reasonable further study of the taxonomic structure of the community at the genus level. This will simplify the comparison of microbial profiling data with the data of classical microbiology.

(3) Among chemical factors, the concentration of nitrates exerts an impact on the shares of many dominant families, while the concentrations of potassium and ammonium in the analyzed ranges do not significantly affect them. It is important that chemical factors in the real ranges may not affect taxa, which theoretically should noticeably depend on them, as in the case of ammonium concentrations and the proportion of the Nitrosomonadaceae family. This result proves the complicated transition from cultural characteristics to ecological ones and the urgency of collecting data on the ecology of microorganisms under natural conditions or in experimental conditions close to the natural ones.

It is important to search for experimental approaches for ecological testing of complex microbial communities and for the study of the effect of the chosen factors on their structure. The application of such method should enable particular species or groups within the community to show those adaptive abilities that are important under natural conditions. The method's evaluation should be based on the ecological practicability. Our results comprise the necessary basis to determine the boundaries of community variability under natural conditions and a list of the most important taxa, the successful development of which may become a criterion of the adequate experimental models.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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