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SOIL BIOLOGY

A Comparative Analysis of Microbiomes in Natural and Anthropogenically Disturbed Soils of Northwestern Kazakhstan

E. V. Pershina[a, b], E. A. Ivanova[a], A. G. Nagieva[c], A. T. Zhiengaliev[c], E. L. Chirak[a], E. E. Andronov[a, b], and N. Kh. Sergaliev[c]

[a]*All-Russia Research Institute of Agricultural Microbiology, Pushkin 8, St. Petersburg 196608, Russia*
[b]*St. Petersburg State University, St. Petersburg 199034, Russia*
[c]*Zhangir Khan West Kazakhstan Agrotechnical University, Uralsk 090009, Kazakhstan*
e-mail: microbioliza@gmail.com

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Abstract—The goal of this study was to determine the relationships between the structure of the soil microbiome and the agroecological state of soils by the example of natural undisturbed (steppe areas) and anthropogenically disturbed (pastures, croplands, fallows) areas in the territory of northwestern Kazakhstan. The highest abundance of proteobacteria was found in the anthropogenically disturbed of fallows and in undisturbed soils; in other cases, actinobacteria and representatives of the *Firmicutes* phylum predominated. Different kinds of anthropogenic impacts resulted in the decrease in the portions of bacteria from the *Acidobacteria*, *Gemmatimonadetes*, and *Firmicutes* phyla. In the disturbed soils, the portions of bacteria from the *Erysipelothrix, Mycobacterium, Methylibium, Skermanella, Ralstonia, Lactococcus, Bdellovibrio, Candidatus nitrososphaera, Catellatospora, Cellulomonas, Stenotrophomonas,* and *Steroidobacter* genera increased. Bacteria of the *Erysipelothrix* and *Methylibium* genera occurred only in the undisturbed soils. The anthropogenically disturbed and undisturbed soils differed significantly in the taxonomic structure of their microbiomes forming two separate clusters, which confirms the efficiency of using the data on the structure of soil microbiomes when assessing the agroecological status of soils.

Keywords: soil microbiome, 16S rRNA, highly efficient sequencing, disturbed agrolandscapes, microbiological monitoring

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INTRODUCTION

For the last twenty years, in soil microbiology, a true revolution related to understanding the real scales of biological diversity of prokaryotes in soils of different types has occurred [11, 14, 15], mainly due to the tremendous growth and implementation of molecular biology methods. Currently, works in the field of molecular ecology of microorganisms are unified under the common name "metagenomic investigations." The analysis of soil DNA has confirmed the presence of a great number of uncultivated microorganisms in soils. Their portion can be up to 90% of the community composition [16, 28]. It turned out that the soil metagenomes contained a huge amount of genetic information (1 g of soil may contain 10^{15}–10^{16} pairs of DNA nucleotides corresponding to about 10^9–10^{10} of bacterial genomes). The qualitative analysis of the taxonomic composition of the soil community based on studying the 16S rRNA gene showed that the number of species forming a microbial community amounted to thousands [32]. Right now, prospects of applying the molecular–genetic methods in soil microbiology become evident. Among them is the more complete investigation of the soil genome, including studies of the properties of both cultivated and uncultivated microorganisms, the determination of the composition and functions of soil microbial associations, as well as of the volume and functional load of the soil microbiological and genetic potentials [4, 25].

The changes in the methodological approaches to the investigation of soil microorganisms will allow proposing a modern solution of such a global problem as the degradation of lands in the course of their agricultural use [24]. There is a number of foreign scientific papers devoted to the influence of anthropogenic factors (cattle grazing [19, 29], plowing for crops [17, 18, 22, 24, 27]) on the composition of the soil microbiome. The use of lands as pastures was shown to change the microbiota composition towards the predominance of the *Actinobacteria* phylum (the *Solirubrobacterales* and *Actinomycetales* phyla, in particular [29]), whereas if the lands were used for growing crops, proteobacteria from the *Betaproteobacteria* and *Gammaproteobacteria* phyla became dominant ones [20]. The more detailed analysis for the structure of the soil microbiome has revealed genera of microor-

ganisms associated with the disturbed agrolandscapes; first of all, these are the *Blastococcus, Microlunatus, Pseudonocardia, Solirubrobacter, Brevundimonas, Pseudomonas, Stenotrophomonas, Sphingomonas,* and *Rhodoplanes* genera [16, 17].

The data presented show that the soil microbiome is an indispensable natural object for analyzing the agroecological state of soil owing to its high diversity and the ecological diversification associated with it. The main advantage of this analytic method, unlike traditional approaches (analysis of soil physicochemical characteristics), is the possibility to conduct monitoring of such difficultly formalized biological properties of the soil cover as fertility, soil exhaustion, the total effect of anthropogenic factors, etc.

In Russia, the relationships between the characteristics of the microbiome and soil fertility have not been studied properly. There are some works related to the characterization of the main physicochemical parameters determining the composition of natural communities of soil microorganisms [6] and to the detection of a response of the soil microbiome to the influence of stress factors, namely, the application of fertilizers [2, 3] and salinity [1, 4]. An experience of the international scientific school showed that the scope of metagenomic studies, including the greater number of soil samples and their deeper analysis, should be significantly increased. Particularly, in Europe, at least two large scientific consortia, Terragenome and GenoSol [26, 32], study the problem of soil microbiome. In order to fill the gap in the knowledge of this problem, within a program on collection of samples and their microbiological analysis organized by the All-Russian Research Institute of Agricultural Microbiology, forty samples of soils under different anthropogenic loads were collected from undisturbed natural (steppe) and anthropogenically disturbed (pastures, croplands, fallows) areas in Northwestern Kazakhstan. The main aim of this work was a search for relationships between the structure of the soil microbiome and agroecological state of soils.

OBJECTS AND METHODS

To implement the objective of the work, a representative collection of soil samples reflecting the diversity of natural soils in West Kazakhstan region and the diversity of anthropogenically disturbed soils (cropland, pasture, hayfield, and fallow) was gathered (Fig. 1). A total of six main soil types were investigated: chestnut, meadow, brown, chernozem, solonetz, and solonchak (Table 1). The soil samples (40 in total) were taken from the depths of 0 to 10 cm and 10 to 20 cm. In all the plots, descriptions of vegetation (Table 1) were made, and the main agrochemical characteristics of the soils were determined (Table 2).

The DNA isolation was carried out using a PowerSoil® DNA Isolation Kit (MO BIO, USA), which included abrasive materials for the mechanical destruction of soil samples (Mobio Laboratories, USA). The soil was destroyed using a Precellys 24 (Bertin Technologies, France) homogenizer. The DNA amount and the purity of its extraction were checked by electrophoresis in 1% agarose in × 0.5 TAE buffer. The mean DNA concentration in a sample was 50 ng/mL.

For the purification of DNA, electrophoresis of the extract obtained at the previous stage of its isolation was made in × 0.5 TAE buffer. A cut block containing DNA was placed in a 1.5-mL tube, and two volumes (approximately calculated by the weight of the agarose block) of solution I (3M guanidine isothiocyanate, 20 mM TRIS-HCl, 20 mg/mL of Triton X-100, pH 7.0) were added there. The obtained material was incubated at 65°C up to its complete dissolution. To the obtained solution (the approximate volume is 0.5 mL), 20 μL of solution II (fine-dispersed silicon oxide suspended in solution I up to the final concentration of 40 mg/mL) was added; it was mixed and incubated for 5 min at room temperature, periodically shaking on a rotary shaker. Then, the material was centrifuged at the maximum rate, and the supernatant was completely removed. The sediment was re-suspended in 200 μL of solution III (25 vol % ethanol, 25 vol % isopropanol, 100 mmol NaCl, 10 mmol TRIS-HCl, pH 8.0), centrifuged, and the supernatant was completely removed; the sediment was re-suspended in ethanol once more, centrifuged, and the supernatant was fully removed. After this procedure, the sediment was dried in the air for 15 min, 50 μL of elution buffer (10 mmol TRIS-HCl, 1 mmol EDTA, pH 8.0) were added and eluted using a vortex (under moderate shaking) for 20–30 min. Then, it was centrifuged, and the supernatant was removed.

The purified DNA preparation was used as a matrix in the PCR reaction with universal primers to the variable V4 site of the 16S rRNA gene, F515 GTGCCAGCMGCCGCGGTAA and R806 GGACTACVSGGGTATCTAAT [1], with the addition of oligonucleotide identifiers for each sample and service sequences necessary for pyrosequencing. The primers used were constructed based on analyzing the nucleotide sequences of both bacteria and archaea. They permit amplification of a fragment (400 p.n. long) of the 16S rRNA gene. The preparation of the samples and sequencing were carried out using the GS Junior (Roche, USA) device according to the recommendations of the producer.

The data were processed according to the QIIME 1.8.0 program [10]. The processing of the sequenced sequences of the 16S rRNA gene was accomplished by several stages. At the first stage, the quality of sequences was tested: sequences with a length of less than 200 nucleotides, those having the quality score less than 25, and sequences containing incorrectly read sequences of primers and multiplex identifiers

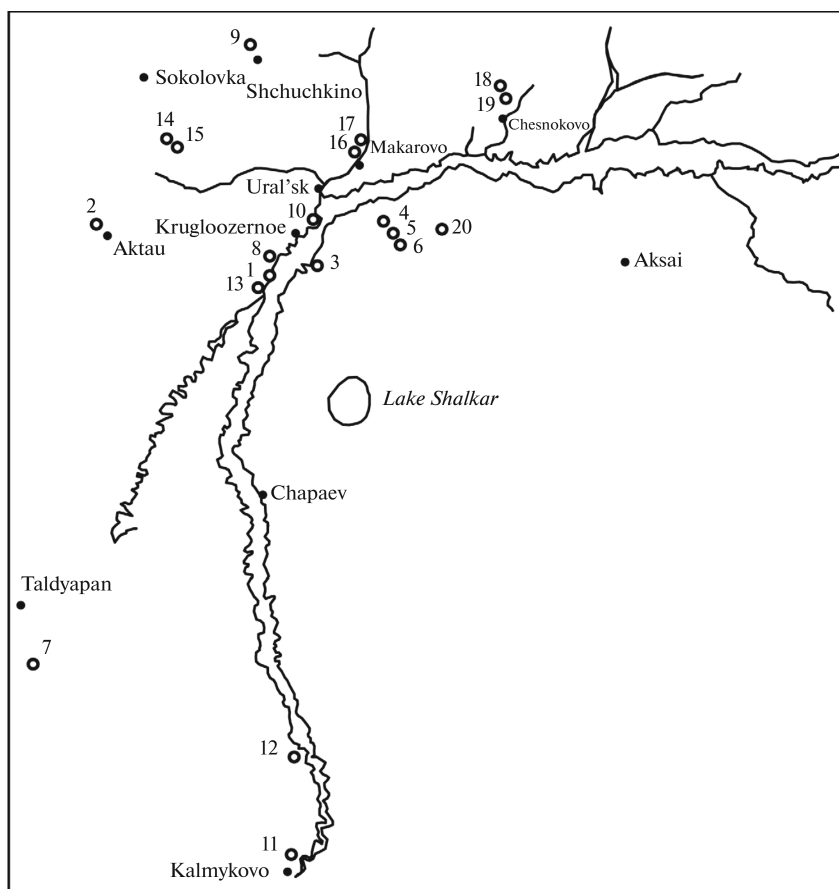

Fig. 1. A scheme of localities for soil sampling in the valley of the middle reaches of the Ural River (Northwestern Kazakhstan): 1–10—undisturbed, 11–20—anthropogenically disturbed soils.

were excluded, as well as extended homopolymeric repeats (more than 8 nucleotides) and unidentified nucleotides. All the non-bacterial and chimeric sequences were removed from the libraries, and the latter were normalized according to the number of smaller sequences. As a result of the procedures carried out, 49080 sequences (1227 sequences in each library) were selected. The sequences with similarity exceeding 97% were combined into operational taxonomic units (OTUs) using the *de novo* algorithm (the basis is the "uclust" method). From each OUT, one sequence was selected for making a set of representatives. At the next stage, a classification of representative sequences was accomplished using the RDP nave Bayesian rRNA Classifier and the PyNast algorithm equalization [10]. A specially constructed set of sequences—"Greengenes coreset"— was used as a matrix [13]. The equalized sequences were used for constructing a matrix of genetic distances and a phylogenetic tree.

For the assessment of biodiversity and comparison of communities, parameters of α- and β-diversity were calculated. The α-diversity was estimated using indices of species richness (the number of OUTs in a sample) and the Shannon index. The significance of differences between the microbiomes according to indices of α-diversity was assessed using *t*-test. For the estimation of β-diversity, the "weighted unifrac" method was applied. This method allows determining the percentage of similarities/differences between all the pairs of the microbiomes compared [21]. The results were processed using methods of multivariate statistics (analysis of principal components) in the Emperor program.

For determining the taxa of microorganisms by significantly changing their abundance under the influence of some factor, the ANOVA test (Statistica10 Enterprise) was used. The Mantel statistical test (1000 permutations) was applied to assess the effect of agrochemical characteristics on the composition of the soil microbiome.

RESULTS AND DISCUSSION

The analysis of the taxonomic structure of the soil microbiomes has revealed 29 bacterial and 2 Archean phyla, among which representatives of 11 phyla predominated (%) (*Crenarchaeota*—15, *Acidobacteria*—15, *Actinobacteria*—54, *Bacteroidetes*—15, *Chloroflexi*—6,

Table 1. Characteristics of the soil samples

Sample number	Soil type, land	Position	Plant community, projective coverage, dominant species
		Disturbed soils	
1.1, 1.2*	Chestnut solonetz	31 m a.s.l., 50°56′52.14″ N, 51°7′56.58″ E	Wormwood–gramineous, 85–90%, *Poa bulbosa, Androsac Turczaninovii* Freyn, *Stipa Lessingiana* Trin. *Eremopyrum orientale, Bromus tertorum*
2.1, 2.2	Dark chestnut incompletely developed	86 m a.s.l., 51° 0′9.00″ N, 50° 9′15.00″ E	Wormwood–gramineous, 75–85%, *Poa bulbosa, Androsac Turczaninovii* Freyn, *Stipa Lessingiana, Eremopyrum orientale, Bromus tertorum*
3.1, 3.2	Dark chestnut weakly developed	105 m a.s.l., 51°0′31.92″ N, 51°18′16.68″ E	Wormwood–gramineous, 85–90%, *Poa bulbosa, Androsac Turczaninovii* Freyn, *Stipa Lessingiana* Trin. *Eremopyrum orientale, Bromus tertorum*
4.1, 4.2	Solonchak	53 m a.s.l., 51°5′28.38″ N, 51°42′30.90″ E	Weeds, 45–60%, *Thlaspi arvense, Lactuca tatarica, Capsella bursa-pastoris, Convolvulus arvensis*
5.1, 5.2	Meadow-chestnut	48 m a.s.l., 51° 8′46.44″ N, 51°39′14.04″ E	Hydrophilous forbs, 90–95%, *Poa bulbosa, Salvia tesquicola, Euphorbia virgata, Juncus gerardi*
6.1, 6.2	Dark chestnut medium thick	52 m a.s.l., 51°6′49.38″ N, 51°39′41.64″ E	Hydrophilous forbs and mesophytes, 75–80%, *Festuca valesiaca, Poa bulbosa, Euphorbia virgata, Jurinea multiflora*
7.1, 7.2	Light chestnut	9 m a.s.l., 49°33′27.00″ N, 50°16′14.94″ E	Xerophytes, 90–95%, *Thlaspi arvense, Lactuca tatarica, Capsella bursa-pastoris, Convolvulus arvensis, Limonium gmelinii, Ceratocephalus ortho-ceras, Petrosimonia oppositifolia*
8.1, 8.2	Chestnut	29 m a.s.l., 50°52′35.94″ N, 51°6′29.34″ E	Gramineous–herbs communities with dominant graminoids, 90–95%, *Artemisia sp., Stipa capillata, Glycyrrhiza sp., Agropyron cristatum*
9.1, 9.2	Southern chernozem	148 m a.s.l., 51°40′18.06″ N, 50°48'53.94″ E	Hydrophilous forbs and mesophytes, 7–80%, *Festuca valesiaca, Poa bulbosa, Euphorbia virgata, Jurinea multiflora.*
10.1, 10.2	Floodplain chestnut	32 m a.s.l., 51°7′43.86″ N, 51°21'50.88″ E	Wood vegetation, *Ulmus angustifolia*
		Anthropogenically disturbed soils	
11.1, 11.2	Solonetzic brown loamy-clayey, pasture,	–1 m a.s.l., 49°3′51.78″ N, 51°49′31.08″ E	Wormwood–gramineous, 35–40%, *Tulipa schrenkii,Thlaspi arvense, Poa bulbosa, Eremopyrum* sp., *Androsace maxima, Stipa capillata, S. pennata, Festuca valesiaca, Gypsophila paniculata, Lepidium perfoliatum, Chenopodium album, Artemisia austriaca, Achillea millefolium*.
12.1, 12.2	Light chestnut loamy-clayey, pasture (former irrigated cropland)	2 m a.s.l., 49°23′25.86″ N, 51°41′57.54″ E	Wormwood–gramineous, 30–35%, *Poa bulbosa, Eremopyrum sp., Androsace maxima, Stipa lessingiana, S. pennata, Festuca valesiaca, Gypsophila paniculata, Lepidium perfoliatum, Chenopodium album, Atriplex oblongifolia*
13.1, 13.2	Chestnut loamy-clayey, irrigated cropland without application of manure and fertilizers	24 m a.s.l., 50°52′52.02″ N, 51°6′52.14″ E	Herbs–feather grass, 45–60%, *Poa bulbosa, Eremopyrum, Stipa capillata, S. pennata, Elytrigia repens, Festuca valesiaca, Androsace maxima, Gypsophila paniculata, Lepidium perfoliatum, Chenopodium album, Bassia hirsuta, Atriplex oblongifolia, Ceratocarpus arenarius, Artemisia austriaca, A. absinthium, Achillea millefolium, Tanacetum millefolium, Chorispora sp., Xanthium strumarium, Convolvulus arvensis, Euphorbia seguieriana, Erysimum canescens*

Table 1. (Contd.)

Sample number	Soil type, land	Position	Plant community, projective coverage, dominant species
14.1, 14.2	Dark chestnut shallow loamy-clayey, fallow (long used as a cropland)	150 m a.s.l., 51°16′2.04″ N, 50°28′47.82″ E	Wormwood–spurge, 25–30%, *Poa bulbosa, Eremopyrum, Anisantha tectorum, Androsace maxima, Lepidium perfoliatum, Artemisia lercheana, A. absinthium, Chorispora, Convolvulus arvensis, Euphorbia seguieriana, Erysimum canescens*
15.1, 15.2	Dark chestnut incompletely developed loamy-clayey, fallow	138 m a.s.l., 51°16′26.40″ N, 50°29′26.40″ E	Wormwood–spurge, 25–30%, *Poa bulbosa, Eremopyrum, Anisantha tectorum, Androsace maxima, Lepidium perfoliatum, Artemisia lercheana, A. absinthium, Chorispora, Convolvulus arvensis, Euphorbia seguieriana, Erysimum canescens*
16.1, 16.2	Meadow chestnut ordinary loamy-clayey, spring pasture, Periodically flooded	33 m a.s.l., 51°22′32.64″ N, 51°29′0.96″ E	Herbs–feather grass, 55–60%, *Poa bulbosa, Eremopyrum, Stipa capillata, S. pennata, Festuca valesiaca, Androsace maxima, Lepidium perfoliatum, Chenopodium album, Bassia hirsute, Atriplex oblongifolia, Artemisia austriaca, A. absinthium, Achillea millefolium, Tanacetum millefolium, Convolvulus arvensis, Euphorbia seguieriana, Salvia stepposa, Medicago falcate, Trifolium repens, Plantago stepposa*
17.1, 17.2	Meadow, ordinary, loamy-clayey, medium thick, hayfield, grazing, signs of the influence of groundwater and surface moistening	33 m a.s.l., 51°22′32.64″ N, 51°29′0.96″ E	Herbs–feather grass, 50–65%, *Poa bulbosa, Eremopyrum, Stipa capillata, S. pennata, Festuca valesiaca, Agropyron cristatum, Androsace maxima, Lepidium perfoliatum, Atriplex oblongifolia, Artemisia austriaca, A. absinthium, Achillea millefolium, Tanacetum millefolium, Serratula erucifolia, Convolvulus arvensis, Euphorbia seguieriana, Salvia stepposa, Medicago falcate, Trifolium repens, Plantago stepposa, Spiraea hypericifolia, Thalictrum simplex*
18.1, 18.2	Southern chernozem, strongly eroded, loamy-clayey, cropland (for a long time)	60 m a.s.l., 51°38′48.18″ N, 52°12′11.34″ E	Herbs–wheat grass, 25–30%, *Eremopyrum, Convolvulus arvensis, Euphorbia seguieriana, Lactuca tatarica, Thlaspi arvense*
19.1, 19.2	Dark chestnut strongly eroded, loamy-clayey, fallow (used in agriculture for more than 40 years)	60 m a.s.l., 51°38′48.60″ N, 52°12′10.50″ E	Herbs–wheat grass, 35–40%, *Agropyron cristatum, Anisantha tectorum, Androsace maxima, Convolvulus arvensis, Euphorbia seguieriana, Lactuca tatarica, Thlaspi arvense, Capsella bursa-pastoris*
20.1, 20.2	Chestnut solonetz, medium loamy-clayey, cropland (used in agriculture for more than 40 years)	79 m a.s.l., 51°11′9.42″ N, 51°58′39.60″ E	Wormwood–herbs, 65–70%, *Agropyron cristatum, Anisantha tectorum, Koeleria glauca, Festuca valesiaca, Stipa capillata, S. pennata, Androsace maxima, Euphorbia seguieriana, Artemisia austriaca, A. lercheana, Achillea millefolium, Tanacetum millefolium, Salvia stepposa, Camphorosma monspeliaca, Spiraea hypericifolia*

In the sample number, after the dots, 1 and 2 designate the layer of soil sampling: 0–10 and 10–20 cm, respectively.

Firmicutes–29, *Gemmatimonadetes*–9, *Planctomycetes*–3, *Nitrospira*–2, *Proteobacteria*–71, and *Verrucomicrobia*–15). The maximum abundance of proteobacteria was observed in the fallow (14.1 and 14.2) and undisturbed soils (9.2, 10.1, 10.2); in some other cases, actinobacteria were dominant, except for sample 6.1, where the high abundance of bacteria from the *Firmicutes* phyla was found (Fig. 2).

By the data of the ANOVA test (Table 3), in the undisturbed soils, unlike the anthropogenically dis-

Table 2. The results of the agrochemical analysis of soils

Sample number	Depth, cm	$N-NO_3$, mg/kg	P_{mobile}	K_{mobile}	Humus, %	pH H_2O	Composition of water extract, cmol(equiv)/kg				Mg^{2+}	Na^+	Sum of salts, %
			cmol/kg				HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	cmol(equiv)/kg		
Undisturbed soils													
1	0–18	14	1.5	23.6	1.90	8.38	0.68	2.49	0.11	0.53	0.62	2.13	0.20
2	0–15	12	5.4	32.1	3.60	8.69	0.32	3.08	0.04	1.53	0.32	1.59	0.20
3	0–10	22	4.4	36.8	3.81	8.84	1.01	2.72	0.14	1.34	0.08	2.45	0.23
4	0–15	14	2.4	25.6	2.96	8.64	0.13	12.55	15.3	15.69	3.24	9.05	1.71
5	1–10	15	10.2	40.2	3.25	8.95	0.49	0.19	1.05	0.24	0.05	1.44	0.26
6	0–10	12	3.5	40.8	2.54	8.06	0.23	0.12	1.84	0.61	0.27	1.31	0.27
7	0–16	15	2.2	50.2	1.62	9.61	0.52	2.63	0.02	1.81	0.77	0.59	0.18
8	0–15	17	2.5	45.2	2.05	8.33	0.49	0.76	0.05	0.66	0.18	0.46	0.08
9	0–15	12	7.2	48.4	3.74	8.37	0.04	0.00	0.08	0.00	0.00	0.04	0.16
10	10–30	15	15.2	62.2	3.91	8.41	0.47	0.05	0.03	0.35	0.01	0.02	0.04
Anthropogenically disturbed soils													
11	0–16	11	0.7	5.4	1.04	8.63	0.006	0.014	0.007	0.005	0.003	0.003	0.04
12	0–10	15	1.8	15.4	1.50	9.11	0.012	0.009	0.024	0.005	0.002	0.013	0.07
13	0–20	23	2.6	32.4	2.35	8.52	0.018	0.004	0.002	0.005	0.002	0.002	0.03
14	0–12	47	3.7	37.7	2.67	8.73	0.012	0.016	0.007	0.008	0.003	0.004	0.05
15	0–6	45	3.9	35.4	2.82	8.52	0.009	0.011	0.007	0.008	0.002	0.002	0.04
16	0–23	44	3.4	42.0	2.95	8.42	0.015	0.009	0.005	0.005	0.003	0.002	0.04
17	0–10	41	5.7	45.1	3.09	8.34	0.015	0.007	0.002	0.003	0.003	0.003	0.03
18	0–14	41	4.7	47.3	2.81	8.31	0.024	0.009	0.002	0.01	0.002	0.002	0.05
19	0–8	38	2.5	36.1	2.34	8.66	0.061	0.025	0.053	0.005	0.003	0.053	0.20
20	0–20	29	1.8	35.2	2.45	8.51	0.040	0.060	0.005	0.015	0.012	0.016	0.15

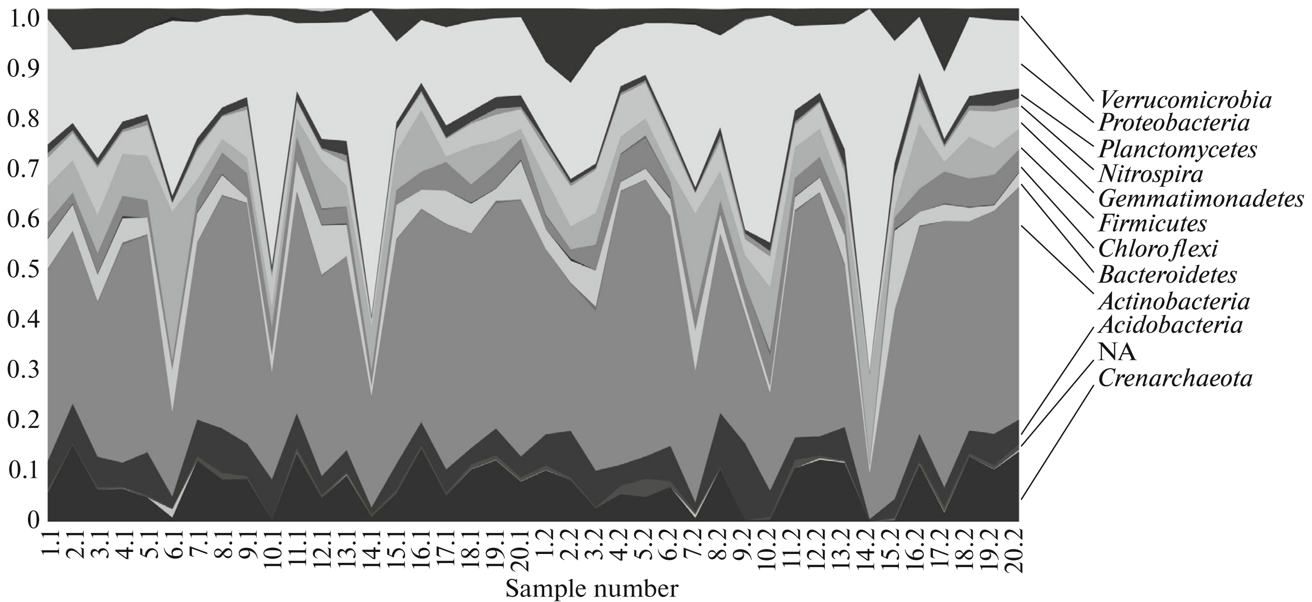

Fig. 2. The taxonomic structure of the microbiomes in the plow (1.1–20.1) and subsurface (1.2–20.2) horizons of the undisturbed (1–10) and anthropogenically disturbed (11–20) soils at the phylum level (only dominant phyla are presented); the relative number of bacterial phyla (in portions) are on the Y-axis.

turbed ones, the content of the *Acidobacteria*, *Gemmatimonadetes*, and *Firmicutes* phyla increased. Literature data show that the representatives of the *Gemmatimonadetes* phylum are capable of adapting to low pressure of soil moisture; they are typical representatives of arid soils [12]. In this case, the increase in the diversity of xerophilic bacteria evidenced the presence of stable (climax) communities in the undisturbed soils as compared to the anthropogenically transformed soils, in which the xerophilic group was represented only by actinobacteria.

The reduction of the diversity in the undisturbed soils was also observed for the *Firmicutes* phyla, which in the disturbed soils was mainly represented by the *Bacilli* class. In the natural soils, clostridia predominated in this phyla (*Clostridia* representatives amounted to 24% of the total composition of the *Firmicutes* phyla). The presence of clostridia testified to additional anaerobic ecological niches in the soil (particularly, the presence of anaerobic zones within soil crumbs). This fact may be an indirect indicator of the aggregate structure and the degree of its development.

In the disturbed soils, the content of the archaen *Crenarchaeota* phylum increased; the majority of its members are extremophiles (hyperthermophiles, in particular [8]) or they can be associated with marine sites [9]. The increase of the extremophilous group in these soils may be an indicator of their ecological instability.

The ANOVA test accomplished for the comparison of the microbiomes in the plow and subsurface horizons has not revealed significant differences in the composition of the known taxa. The microbiomes studied differed only in the composition of unidentified phyla: the abundance of bacteria with the unknown taxonomic position was, on the average, higher in the 10- to 20-cm layer as compared to that in the upper horizon. This fact may be explained by the presence of specialized groups of microorganisms confined to the deeper soil layers, the information of which is absent in databases (in the databases available at the moment, the bacterial sequences isolated from the surface soil horizons (the most popular object for microbiological studies) were mainly represented).

As the taxonomic structure of the microbiome was analyzed at the generic level, 2180 bacterial genera were isolated. The dominant position (>5% of the community composition) belonged to bacteria from the genera of *Clostridiaceae*_NA, *Pseudonocardia*, Ellin6529_NA, *Gemmatimonadetes*_NA, *Corynebacterium*, 0319-7L14_NA (up to 5%), *Geodermatophilaceae*_NA, *Sporosarcina*, *Micrococcales*_NA (6%), *Solirubrobacterales*_NA (7%), *Micrococcales*_NA (8%), *Adhaeribacter* (9%), *Clostridiaceae*_NA (10%), *Bacillales*_NA (10%), koll13_NA (11%), *Actinomycetales*_NA (13%), DA101 (13%), *Micrococcaceae*_NA (15%), *Exiguobacterales*_NA (17%), and *Bacilli*_NA (up to18%). Attention is drawn to the fact that no halophilic bacteria in the solonetzic soils and solonchaks (samples 1, 4, 11, 20) were found among the dominant groups.

In the course of the analysis of the surface (0–10 cm) soil horizons, 940 OUTs were found. They occurred in the anthropogenically disturbed and undisturbed

Table 3. The results of the variance analysis (ANOVA) for the comparison of the taxonomic composition of bacteria at the phylum level for undisturbed and anthropogenically disturbed soils and soil samples taken at different depths

Genus	Comparison of undisturbed and disturbed soils		Comparison of 0–10- and 10–20-cm layers	
	F	*P*	*F*	*P*
Proteobacteria	0.23	0.63	0.09	0.77
Actinobacteria	0.25	0.62	1.24	0.27
Chloroflexi	0.38	0.54	0.14	0.71
Acidobacteria	**14.48**	**0.00**	0.01	0.91
Gemmatimonadetes	**8.65**	**0.01**	1.88	0.18
Bacteroidetes	0.09	0.77	1.12	0.30
Armatimonadetes	0.04	0.84	1.53	0.22
Verrucomicrobia	0.40	0.53	3.56	0.07
Nitrospirae	0.21	0.65	0.53	0.47
Planctomycetes	**5.18**	**0.03**	0.08	0.78
Chlorobi	1.77	0.19	0.05	0.83
NA	0.21	0.65	**4.21**	**0.05**
Firmicutes	**4.11**	**0.05**	0.07	0.80
OD1	0.00	1.00	2.11	0.15
Crenarchaeota	**8.37**	**0.01**	0.17	0.68
Fibrobacteres	0.10	0.76	2.60	0.12
Euryarchaeota	0.87	0.36	0.00	1.00
BRC1	0.87	0.36	0.03	0.85
WS3	0.39	0.54	2.57	0.12
WYO	0.43	0.52	0.43	0.52
Elusimicrobia	2.53	0.12	0.60	0.44
Tenericutes	1.09	0.30	1.09	0.30
Cyanobacteria	0.94	0.34	0.41	0.52
Thermi	1.85	0.18	0.10	0.75
NKB19	1.00	0.32	1.00	0.32
Chlamydiae	1.34	0.25	0.33	0.57
GAL15	0.00	1.00	0.00	1.00
TM6	1.00	0.32	1.00	0.32

The phyla, between which significant differences were found, are in bold; F is the Fisher criterion at 5% significance level; P is the significance of differences (differences are considered significant at $P < 0.05$); NA is bacteria with unidentified taxonomic position.

soils. Fig. 3 presents the taxonomic structure of these OUTs with the identification at the generic level.

The total number of different OTUs was greater in the undisturbed soils. In these soils, the main part of bacteria was unidentified (65%); bacteria of the DA101, *Pseudomonas*, *Erysipelothrix*, *Microbulbifer*, *Deinococcus*, KSA1, *Candidatus solibacter*, and *Clostridium* genera were represented in a smaller number. Among the minor components (OTUs are less than 10 sequenced sequences per an amplicon library), halophilic and alkaliphilic bacteria confined to solonchaks (*Halomonas*, *Natronomonas*, *Natronincola*) and marine bacteria (*Marinobacter*, *Microbulbifer*, *Gramella*, *Prochlorococcus*) should be noted. In the undisturbed soils, a representative group of bacteria participating in the sulfur cycle (*Desulfosporosinus*, *Thiobacillus*, KSA1) and bacteria of the KSA1 appear to also be halophytes. According to literature data, this group was first found when analyzing the microbiota of black sulfide-rich clay on sea coasts [30].

The species richness of bacteria in anthropogenically disturbed soils was markedly lower; bacteria from the *Hymenobacter*, *Novosphingobium*, *Gemmata,* and *Streptococcus* genera predominated. In these soils, halophytes—bacteria of the *Jeotgalicoccus* genus—were also in the composition of the minor component. These bacteria were first described when the microbiota of marine products was analyzed [33]. However, they are present in sediments of tidal zones, salt lakes, and in soils of coastal areas. The recent data show that

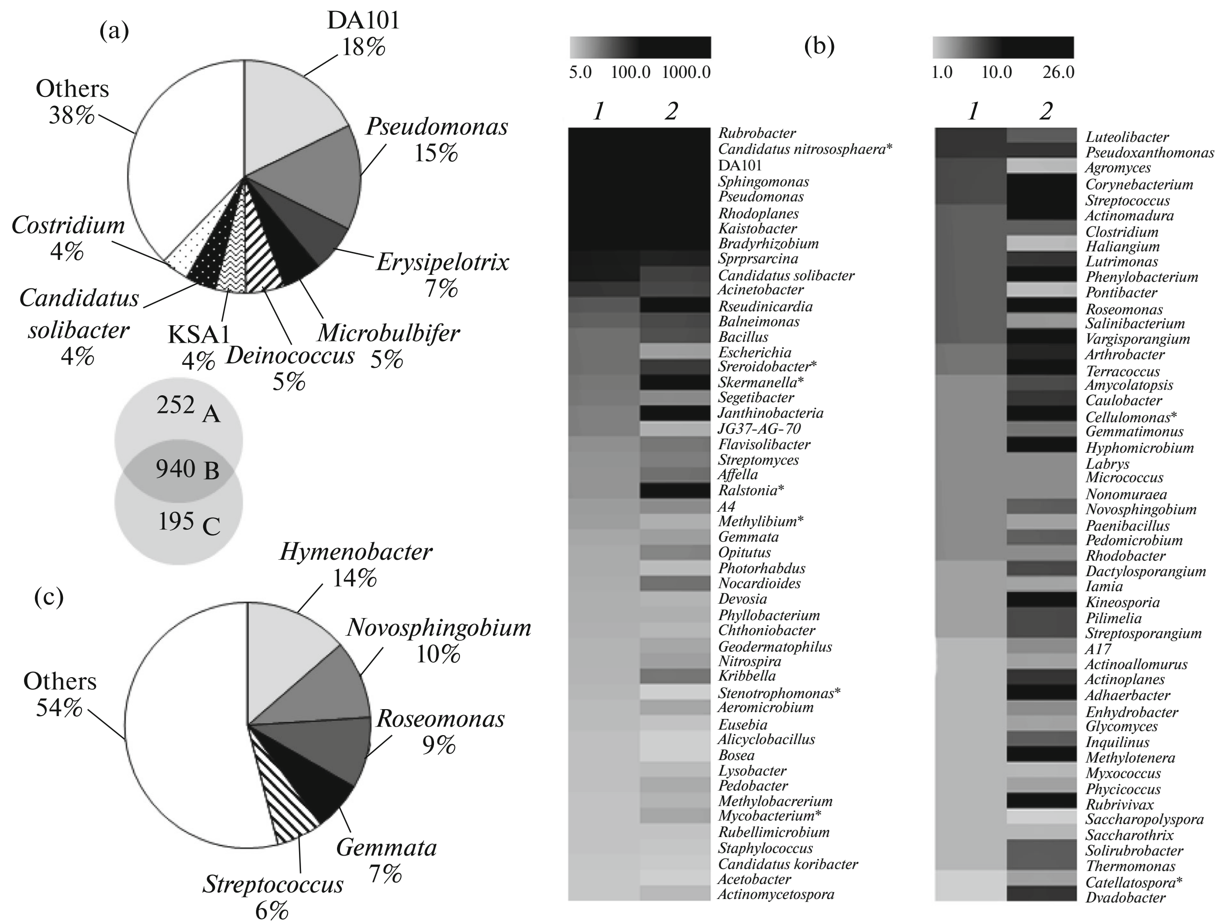

Fig. 3. The comparison of the OUT composition (the taxonomic characteristics are given at the generic level) of the disturbed and undisturbed soils: a—bacteria dwelling only in the undisturbed soils (252 OTUs), b—bacteria occurring in all the soils studied (940 OTUs) (asterisks designate the bacterial genera that significantly differ between the disturbed and undisturbed soils according to the data obtained by the ANOVA test), and c—bacteria inhabiting only anthropogenically disturbed soils (195 OTU); OUT—operational taxonomic unit (a group combining the 16S rRNA genes having 97% of similarity, which approximately corresponds to the taxonomic category of species): *1*—undisturbed soils, *2*—anthropogenically disturbed soils.

this group may be associated with places of domestic animal and bird concentrations [23]. In the soils described, some other halophilic bacteria (*Sphingopyxis* and *Roseomonas*) and groups of actinobacteria confined to arid sites (*Cryptosporangium* and *Kineococcus*) occurred.

The ANOVA test on the generic level has revealed that the microbiomes of the anthropogenically disturbed soils significantly differed from those of the undisturbed soils in the number of bacterial OTUs classified within the *Mycobacterium, Skermanella, Ralstonia, Lactococcus, Bdellovibrio, Candidatus Nitrososphaera, Catellatospora, Cellulomonas, Stenotrophomonas,* and *Steroidobacter* genera (Fig. 3, asterisks). In these soils, the abundance of bacteria from the *Ralstonia, Mycobacterium, Skermanella,* and *Cellulomonas* genera was higher. In the undisturbed soils, the number of *Catellatospora* bacteria significantly increased. Attention is paid to the fact that in the disturbed soils, the portion of bacteria belonging to the group of lower actinobacteria that are biased to copiotrophy increased, whereas in the undisturbed sites, the number of higher actinobacteria (oligotrophs) became higher. In its turn, the predominance of copiotrophic groups is a characteristic feature of disturbed biocenoses.

The analysis of α-diversity (Table 4) showed that all the investigated microbiomes demonstrated the same high level of diversity, except for those in the samples of the fallow soils (14.1, 14.2, and 15.2). The highest species richness was noted in samples 1.1, 6.2, and 17.1. It is of interest that solonetzes and solonchaks also had high α-diversity (relative to that in the other samples), although it is traditionally accepted that these soils are poor in the bacterial species. At the same time, all the differences observed in the α-diversity characteristics can be caused by accidental reasons, since the data of the test performed have not revealed significant differences in the indices of species richness and Shannon index.

Table 4. Characteristics of α-diversity for the studied microbiomes

Sample number	Number of OTUs	Shannon index	Sample number	Number of OTUs	Shannon index
Undisturbed soils			Disturbed soils		
1.1*	557.5	8.12	11.1	516.0	7.99
1.2	515.2	8.10	11.2	514.5	8.00
2.1	507.1	7.87	12.1	489.2	7.88
2.2	520.9	8.10	12.2	507.0	7.97
3.1	535.7	8.39	13.1	526.1	7.96
3.2	542.5	8.44	13.2	533.2	8.12
4.1	492.0	8.09	14.1	307.9	6.54
4.2	492.6	8.04	14.2	177.6	5.43
5.1	513.2	7.90	15.1	535.1	8.30
5.2	485.3	8.07	15.2	342.0	7.26
6.1	430.1	7.68	16.1	475.1	7.55
6.2	554.9	8.24	16.2	500.2	7.73
7.1	498.0	7.81	17.1	554.1	8.34
7.2	443.4	7.92	17.2	445.5	7.62
8.1	496.4	8.02	18.1	527.7	7.95
8.2	542.3	8.09	18.2	511.1	7.94
9.1	478.7	7.80	19.1	503.7	8.02
9.2	499.4	8.36	19.2	512.6	7.94
10.1	525.2	8.41	20.1	535.7	8.12
10.2	481.5	7.90	20.2	526.0	8.06

The mean indices of α-diversity calculated for 10 random samplings with 2000 sequences.

In the course of analyzing the β-diversity, the correlation of matrices obtained using the "weighted unifrac" algorithm (matrix of distances between microbiomes) with those obtained by analyzing the tables of soil agrochemical characteristics was investigated with the use of the statistical Mantel test. The analysis performed did not reveal any significant correlations between the taxonomic composition of microbiomes in the soil samples and the agrochemical characteristics of the soils studied. Probably, this fact is a result of the well-made sampling, so that the samples did not differ much in their chemical properties (Table 2). Thus, we can suggest that the differences observed in the taxonomic composition of microorganisms are first related to the land use history. This statement is confirmed by the data of the multivariate statistical analysis (Fig. 4), which showed the subdivision of the soil samples into two clusters in

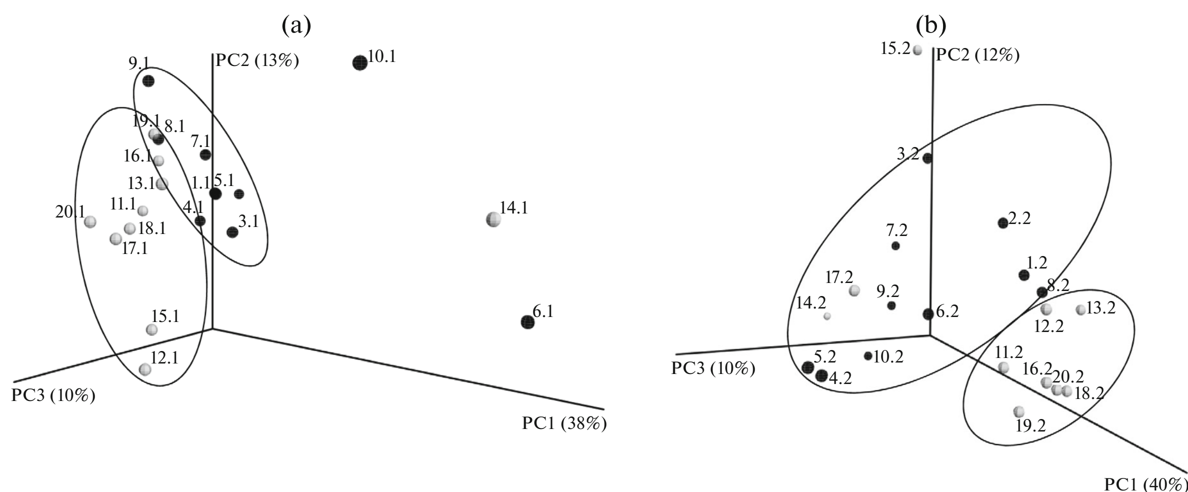

Fig. 4. The analysis of principal components performed for the microbiomes of plow (a) and subsurface (b) horizons of the undisturbed (1–10) and anthropogenically disturbed (1–20) soils; PC1, PC2, PC3 are projections on three principal components, where values of explained variation are given (%).

the variants with the 0–10-cm and 10–20-cm soil layers. The samples of the fallow soils (14.1, 14.2, 15.1, 15.2) had a tendency of grouping with those of the undisturbed soils. This fact shows that the cessation of economic use of the land fast turns the microbial community to the initial state typical for the microbiomes of undisturbed soils.

CONCLUSIONS

The results of the studies based on the investigation of forty samples taken from the 0–10- and 10–20-cm soil layers in Northwestern Kazakhstan showed that the taxonomic structure of the soil microbiomes was determined by anthropogenic impact (plowing of land and using it as pastures) rather than by the soil physicochemical properties.

Anthropogenic impact on soils leads to a decrease in the biodiversity at the phylum and lower taxonomic levels. In the undisturbed and fallow soils, the microbial community is better equilibrated, with the predominance of both actinobacteria characteristic of arid sites and proteobacteria. In the anthropogenically disturbed soils, the equilibrium is shifted towards actinobacteria that become dominants. In the undisturbed soils, the xerophilic group is also more diverse and includes not only actinobacteria, but also uncultivated bacteria from the *Gemmatimonadetes* phylum earlier described.

The increase of biodiversity at the generic level in the undisturbed soils is first related to the high number of bacterial genera that were undescribed (unidentified) earlier and to the more diverse composition of the *Actinobacteria* phylum, which includes oligotrophic members of the group of higher actinobacteria. This fact evidences the stability of the undisturbed ecosystems, as opposed to the disturbed ones, whose soils contain mainly copiotrophic representatives of the *Actinobacteria* phylum.

The investigation carried out showed that the anthropogenically disturbed and undisturbed soils significantly differed in the taxonomic structure of soil microbiomes, which supports the possibility of using data on the structure of soil microbiomes for the assessment of the agroecological status of soils. However, in order to raise the efficiency of the metagenomic approach, it is necessary to study the soil-forming properties of uncultivated microorganisms in more detail, since they play a key role in the maintenance of the stability of biocenoses.

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