AGRICULTURAL BIOLOGY,
2020, V. 55, № 1, pp. 137-152
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Soil microorganisms

UDC 631.618:631.46:577.2

doi: 10.15389/agrobiology.2020.1.137eng
doi: 10.15389/agrobiology.2020.1.137rus

ANALYSIS OF MICROBIOME OF RECULTIVATED SOILS OF THE KINGISEPP AREA OF PHOSPHORITE MINING

A.K. KIMEKLIS[1, 2], Ya.A. DMITRAKOVA[1], E.A. PERSHINA[1, 2], E.A. IVANOVA[1, 2, 3], A.O. ZVEREV[1, 2], G.V. GLADKOV[1, 2], A.A. KICHKO[2], E.E. ANDRONOV[1, 2, 4], E.V. ABAKUMOV[1]

[1]*Saint-Petersburg State University,* 7-9, Universitetskaya nab., St. Petersburg, 199034 Russia, e mail kimeklis@gmail.com (✉ corresponding author), dmitrakovay.a@gmail.com, microbioliza@gmail.com, ektrnivanova@gmail.com, azver.bio@gmail.com, ruginodis@gmail.com, eeandr@gmail.com, e_abakumov@mail.ru;
[2]*All-Russian Research Institute for Agricultural Microbiology,* 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail 2014arki@gmail.com;
[3]*Agrophysical Research Institute*, 14, Grazhdanskii pr., St. Petersburg, 195220 Russia;
[4]*Dokuchaev Soil Science Institute,* 7/str. 2, Pyzhyovskii per., Moscow, 119017 Russia
ORCID:
Kimeklis A.K. orcid.org/0000-0003-0348-7021
Dmitrakova Ya.A. orcid.org/0000-0002-6980-4854
Pershina E.A. orcid.org/0000-0003-0834-3211
Ivanova E.A. orcid.org/0000-0003-1589-9875
Zverev A.O. orcid.org/0000-0002-5315-8632
Gladkov G.V. orcid.org/0000-0002-5248-9018
Kichko A.A. orcid.org/0000-0002-8482-6226
Andronov E.E. orcid.org/0000-0002-5204-262X
Abakumov E.V. orcid.org/0000-0002-5248-9018

The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Science Foundation, grant No. 17-16-01030
Received May 15, 2019

Abstract

The microbial composition of reclaimed disturbed soil covers may indicate the degree of their recovery and the processes occurring in them, as well as their suitability for further use in agriculture. Kingisepp phosphorite quarry was developed in the 1960s, and at the end of the 1970s reclamation was performed. This object is unique because its soil physical parameters were monitored for 29 years and the reclamation was performed with the planting of three plant cultures — spruce, larch and pine. In the area with spruce it was leveled with an addition of peaty-mineral mixture, and in areas with larch and pine only mineral substrate without peat was added. However, the analysis of the microbiome composition of the soil cover at the reclamation sites has not yet been carried out. Our study showed that the structure of the studied soil microbiome did not depend on the physicochemical parameters of the soil, the diversity of the soil microbiome did not correlate with the main mineral nutrients, and the dominant plant species did not significantly affect the structure of the microbiome. The aim of the work was to study the microbiome of these sites using high-throughput sequencing of amplicon libraries of the 16S rRNA gene, as well as to search for the connection between the microbiome composition and the type of remediation and physical and chemical parameters of the soil. For three plots, descriptions of vegetation cover and soil cuts were made, and soil samples were taken to determine their physical and chemical parameters and DNA extraction. The granulometric composition of the samples, pH levels, substrate induced and basal respiration, as well as the content of organic carbon, mobile compounds of phosphorus and potassium, exchangeable ammonium and nitrates were measured. Quantity of bacteria, archaea and fungi was determined using real-time PCR. For the analysis of microbial communities, the level of their alpha and beta diversity was measured, their taxonomic structure was determined, as well as their relationship with the soil biochemical parameters and vegetation cover. According to the results of the studies, the soil parameters were similar for all plots, and the levels of basal and substrate-induced respiration were very low (around 0.02-0.05 μg CO_2/g per hour). The plot under the spruce showed a more acidic soil extract reaction (pH 6.5) than the plots under larch and pine (pH 7.6 and 7.1, respectively). The type of vegetation was not a sufficiently strong ecological factor and microbial communities turned out to be close in structure. The quantitative composition of microorganisms did not differ significantly between the three experimental plots, except for the lower content of archaea in the plot with spruce. The level of alpha-diversity of the prokaryotic community in all three plots was also

similar, but the area under the spruce differed from others by a higher diversity of actinobacteria. *Proteobacteria*, *Actinobacteria* and *Acidobacteria* phyla were dominant in all samples. The most numerous taxon in all plots was *Pseudomonas*, in the plot with spruce dominated *Actinobacteria*, *Rhizobiaceae*, *Kouleothrixaceae*, Ellin6529, N1423WL, with pine — *Rhodoplanes* and *Sinobacteraceae*, with larch — IS-44. Pine plot was also characterized by a relative low content of *Micrococcaceae* and Ellin6075, and spruce plot — of RB41. In general, in the studied microbiomes, bacteria are identified that belong to both oligotrophic slow-growing forms characteristic of stabilized soil communities with a full carbon cycle, and to fast-growing copyotrophic, often associated with a rhizosphere niche. In this regard, this stage of overgrowing of reclaimed soils of the Kingisepp phosphorite deposit can be attributed to pre-climax.

Keywords: reclamation, soil microbiome, alpha and beta diversity, high throughput sequencing, 16S rRNA

The widespread increase in territories with disturbed soil and plant cover which require land reclamation is an urgent environmental problem of our time. The formed mining complexes need restoration and costly reclamation, at the same time they are good sites for studies of soil formation and primary succession processes [1]. Such observations are important in the applied aspect, since reclaimed soils can again acquire agricultural significance [2], and in fundamental aspects, the study of pedogenesis will allow us to approach understanding of soil evolution.

As a rule, during restoration of ecosystems, the main attention is paid to studying the physicochemical parameters of soils [3] or to vegetation cover [4, 5]. However, the soil microbiome remains the key factor in soil formation processes [6, 7]. The widest understanding of the microbiome is provided by high-throughput sequencing methods [8-10], which allow the most complete description of the microbial composition of samples [11]. In this case, the composition of the microbiome can vary to a significant extent depending on various biotic and abiotic influences [12]. Soil moisture and pH are among the strongest factors affecting soil microbiomes [13, 14]. An important role is given to the diversity of the vegetation cover [15-17]. In particular, we previously showed that with a relatively small scatter in the values of physicochemical parameters within the same soil type, the formation of a certain soil microbiome profile is largely determined by the structure and composition of phytocenosis [18], with differences being observed mainly at a low taxonomic level (orders, families and genera).

Thus, the issue on how the rehabilitation of the soil cover occurs and how its microbiome changes in this case remains relevant. At present, the formation and diversity of microbial communities in disturbed territories is largely associated precisely with the decisive role of plant communities [19]. Since the rehabilitation of disturbed soil cover takes a long time, for soil studies it is necessary to choose sites that are under long-term monitoring. An example is Kingisepp quarry, where phosphate rock was mined in the 1960s, and mining reclamation was carried out in the late 1970s. The uniqueness of this object is that the monitoring of soil indicators has been carried out here for 29 years, and the restoration was carried out using three different tree species [20]. However, no microbial composition analysis has yet been carried out at the reclamation sites, which would make it possible to assess the degree of restoration of the soil cover.

In the present paper, we showed that in the reclamation areas of the Kingisepp phosphorite deposit, the structure of the soil microbiome did not depend on the physicochemical parameters of the soil, the diversity of the soil microbiome did not correlate with the main mineral nutrients, and the dominant plant species did not significantly affect the microbiome profile.

The aim of our work was profiling microbiomes of reclaimed soils and to search for patterns that link the microbiome structure with physicochemical soil

parameters and the dominants of woody vegetation.

Materials and methods. The surveys were carried out in 2016 in quarry No. 3 of Phosphorit Production Association (Kingisepp phosphorite deposit, west of the Leningrad Region, Kingisepp District, between Kingisepp and Ivangorod). Samples were collected at three 20×40 m sites (Nos. 1, 2 and 3) subjected to restoration in the 1970s. The age of the test plots was 37, 32, and 28 years, respectively. In 2014, geobotanical descriptions of the sites were carried out according to the dominant scheme; in 2016, studied the recent (new) soil formation was studied.

At each site, 3 soil sections were laid. When describing soil profiles, a substantive-morphological approach was used, based on the identification of soil taxa by the totality of morphological characters. From each horizon, three probes were taken, 100 g each, for laboratory analyzes. A 15 g sample from each upper organomineral horizon was used for microbiological analysis.

The particle size distribution was determined using the Kaczynski pipetting method with pyrophosphate peptization of microaggregates [21], the organic carbon content was determined by Tyurin's method based on dichromate oxidizability [22], and the pH of the aqueous extract was determined potentiometrically in a soil:water ratio of 1:2.5. The concentration of mobile phosphorus and potassium compounds was measured as per Kirsanov in TsINAO modification [23], exchangeable ammonium by TsINAO method [24], and nitrates ionometrically [25]. The substrate-induced respiration (SIR, the measurement is based on the registration of additional CO_2 emission in response to glucose as the introduced substrate) and basal respiration (BR, determined by the same method as SID, but without enriching soil with glucose) was evaluated as described [26].

DNA was extracted in 4 replicates for each plot by previously developed method [27] with glass balls of different diameters as abrasive. Soil samples were homogenized (a Precellys 24 devise, Bertin Technologies, France). The purity and amount of DNA in the preparation was assayed electrophoretically in 1% agarose with 0.5× TAE buffer (DNA concentration in a sample averaged 50 ng/ml). Purified DNA was used for quantitative PCR (qPCR) and preparation of amplicon libraries as per the instructions of the sequencing protocol (Illumina, Inc., USA).

To identify three main taxonomic groups of microorganisms in the soil, quantitative qPCR was performed with the following primer pairs: to the 16S rDNA fragment of bacteria — EUB338 (5′-ACTCCTACGGGAGGCAGCAG-3′) and EUB518 EUB518 (5′-ATTACCGCGGCTGCTGG-3′) [28, 29], to the archaea 16S rDNA fragment — ARC915f (5′-AGGAATTGGCGGGGGAGCAC-3′) and ARC1059r (5′-GCCATGCACCWCCTCT-3′) [30], to the fungal ITS fragment — ITS1f (5′-TCCGTAGGTGAACCTGCGG-3′) и 5.8S (5′-CGCTGCGTTCTTCATCG-3′) [31]. qPCRmix-HS SYBR kit (Eurogen, Russia) was used to prepare the reaction mixture according to the manufacturer's instructions. The standards were the series of 10-fold dilutions of fragments of the 16S rRNA gene of *E. coli* and *H. pilori*, as well as the ITS1 fragment of *S. cerevisiae*. Each sample of the PCR mixture, including standards, was analyzed in triplicate. The measurements were carried out on a CFX96 thermocycler (Bio-Rad, Germany) according to the following protocol: 3 min at 95 °C; 20 s at 95 °C, 20 s at 50 °C, and 20 s at 72 °C (40 cycles). For replicates of both PCR and different DNA samples in a site), the mean values (*M*) and standard errors the mean (±SEM) were calculated. After processing, the results were expressed as the number of ribosomal operons per 1 g soil

In preparing libraries of 16S rRNA gene fragments for each soil DNA sample, PCR was performed with universal primers for the variable region V4, the F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACVSGGGTATCTAAT-3′) (a T100 thermocycler, Bio-Rad, Germany) according to the

following protocol: 3 min at 95 °C; 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C (35 cycles) [32]. Sequencing and primary processing of data was performed (an Illumina MiSeq instrument, (Illumina, Inc., USA) in the ARRIAM Genomic Technologies and Cell Biology Center for Collective Use of Research Equipment. Sequenced fragments of the 16S rRNA gene were processed using the Trimmomatic [33] and QIIME [34] software. All adapter sequences were trimmed, paired-end reads were assembled, and the quality of nucleotide sequences was checked. All non-bacterial and chimeric sequences were removed, and data were normalized. Sequences with more than 97% similarity were combined into operational taxonomic units (OTUs) using the de novo UCLUST-based algorithm [35]. One sequence was selected from each OTU to compile a set of representative sequences. The representative sequences were classified using RDP naïve Bayesian rRNA Classifier program, and then aligned according to PyNast algorithm [36], using the Green-genes coreset database [37] as a matrix. After sequence alignment, distance matrices were constructed within QIIME using the Euclidean distances.

For a comparative analysis of communities, the indices of α- and β-diversity were calculated. α-Diversity was evaluated using species richness indices (the number of OTUs in the sample, Chao 1 abundance index, Faith's PD phylogenetic diversity index) and the Shannon index of diversity H [38-40].

The significance of differences between microbiomes by α-diversity indices was evaluated using Student's *t*-test. To determine β-diversity, the Weighted unifrac method was used, which allows one to estimate the percentage of similarities/differences between all compared pairs of microbiomes [41]. The results were analyzed using multivariate statistics (analysis of the main components) with Emperor software [42]. A Mantel test (Pearson correlation, 100 permutations) for combined replications was performed with QIIME to assess the correlation of bacterial community composition with concentration of basic soil macroelements [43]. To assess the significance of differences between abundance of individual taxa in analyzed samples, a script we wrote using the Mann-Whitney U-test with a significance threshold of 0.05 in Python programming language [44] was applied in addition to the QIIME software.

The paper presents the mean values of indicators (M) and their standard errors (±SEM). The revealed differences were considered statistically significant at $p < 0.05$.

Results. The Kingisepp quarry was intended for mining phosphorites confined to Obolus sandstones of the Upper Ordovician over which limestones and dolomites lie, a little above which there are Middle Devonian clays, marls, sands, and mudstones. Lacustrine-glacial sands and sandy loams, moraine loams and peat are the Upper Quaternary sediments deposits [3]. All these rocks to one degree or another compose quarry dumps. The phosphorite deposit has been developed open pit since the 1960s. As a result, dump areas of different ages were formed, on which, after several years of self-growth and spontaneous subsidence of the rocks, mine reclamation was applied. This territory is one of the largest in the north-west of Russia in terms of area of violation and the scale of remediation work.

During mine technical restoration at site No. 1 in 1979, after leveling the dumps, a peat-mineral mixture (quaternary loamy-sandy rocks with an impurity peat content of 20-30%) was applied to the blocky material, then biological recultivation was used (planting of spruce seedlings). In sites No. 2 and No. 3, after dump leveling, a mineral substrate consisting of the so-called loose rock that does not contain peat impurities was distributed along the surface. Larches were planted in site No. 2, and pines were planted in site No. 3.

The soils of the reclaimed sites are carbo-lithozems. In general, the main soil parameters turned out to be similar in all sites. However, we note that the content of mobile phosphorus and nitrates is more than 2 times higher in site No. 2 under larch compared to similar horizons in other areas (Table 1). The pH of the aqueous soil extract in the site No. 1 (under the spruce) is weakly acidic, while in the rest it is slightly alkaline. As per granulometric composition, replantozems were characterized as medium loams.

1. Soil biochemical and granulometric parameters of various phytocenoses in the sites of long-term restoration (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016)

Parameter	Replantozem		
	peat-mineral mixture under the spruces 37 years, site No. 1	on mineral rock	
		under the larches, 32 years, site No. 2	under the pines, 27 years, site No. 3
Soil horizon type	AY (	AY	AY
Depth, cm	3-18	1-18	1-10
pH_{BWater}	6.5	7.6	7.2
$C_{org.}$, %	2.0	2.4	1.9
N_{total}, %	0.20	0.31	0.46
C/N	11.6	8.9	4.8
N (NH_4), mg/kg	31.1	27.2	27.4
N (NO_3), mg/kg	0.24	0.82	0.37
P (P_2O_5), mg/kg	2043.5	4198.5	1731.5
K (K_2O), mg/kg	338.3	242.7	214.7
Particle fraction, %:			
< 0.001 mm	15.0	22.0	24.3
< 0.01 mm	33.2	36	36

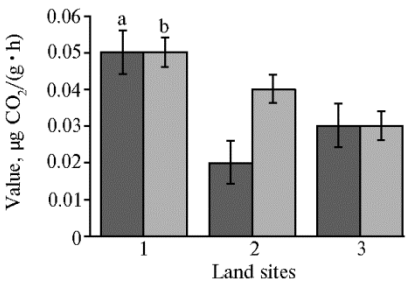

Fig. 1. Basal (a) **and substrate-induced** (b) **soil respiration of organomineral horizons** (AY) **in various phytocenoses at sites of long-term restoration** (n = 3, M±SEM, quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016). For description of sites by type of reclamation, see the section Materials and methods.

In all sites, we revealed very low rates of basal and substrate-induced soil respiration (Fig. 1) with maximum of 0.05 μg CO_2/(g·h) and minimum of 0.02 μg CO_2/(g·h). Additional CO_2 emissions were observed only in site No. 2 (under larch). In general, the soils formed on dumps under pines showed the least respiratory activity. It should be noted that there were no significant differences between the two indicators, although they are usually observed for mature soils of climax ecosystems.

The microbial biomass varied from 1.6±0.1 μg C g (under pines, site No. 3) to 2.4±0.09 μg C g (under spruces, site No. 1). The values of the microbial metabolic coefficient (the ratio of basal respiration to the carbon content of microbial biomass) were extremely small, the maximum value of 0.021±0.002 μg CO_2-C/(mg $C_{mic.}$·h) was also recorded for the soil under spruces, the minimum 0.010±0.005 μg CO_2-C/(mg $C_{mic.}$·h) for soil under larches (site No. 2).

Right after the measures of mining recultivation were applied, self-growth of typical explerents occurred, including *Tussilago farfara* L., *Chamaenerion angustifolium* (L.) Scop., and *Calamagrostis epigeios* (L.) Roth. By 2014, spruce forest was formed in site No. 1, where we identified 11 species of higher vascular plants, 2 ligneous and 9 grassy species, from 8 families. The total projective cover of grassy vegetation here was 5%. In site No. 2 under larches, it reached 25%. In total, 14 species of higher plants from 10 families, 4 ligneous and 10 grassy spe-

cies, grew in site No. 2. In site No. 3 with planted pines, 12 species of higher plants, 3 ligneous and 9 grassy, were revealed at a total projective grass cover of 25%. Approximately the same number of species was accounted for a variety of life forms of herbaceous plants, namely long- and short-rhizomatous plants, tap-root plants, tap-fibrous-root plants, sod perennial grasses. As per the Shannon index H and the inverse Simpson index 1/D (Table 2), the smallest diversity was characteristic of the spruce forest with dead soil cover.

2. Diversity of plant communities in various phytocenoses at sites of long-term restoration (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2014)

Site No.	Shannon index H	Inverse Simpson index 1/D	Number of species	
			per site	total
1	1.3	2.1	11	
2	2.0	4.2	14	22
3	1.9	4.2	12	

N o t e. For description of sites by type of reclamation, see the section Materials and methods.

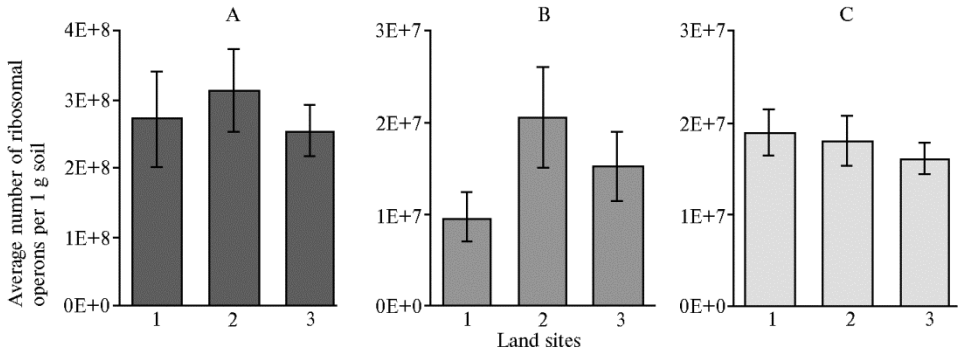

Fig. 2. qPCR-based quantitation of three groups of microorganisms in soil samples from various phytocenoses at sites of long-term restoration: A — bacteria, B — archaea, C — fungi (n = 4 for sites Nos. 1 and 2, n = 3 for site No. 3; 3 measurements for each repetition, M±SEM; quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016). For description of sites by type of reclamation, see the section Materials and methods.

qPCR analysis of abundance of three groups of microorganisms in three test sites revealed some differences in the number of ribosomal operons of bacteria and archaea (Fig. 2). Their counts turned out to be the largest in site No. 2 under larch, $(3.13\pm0.6)\times10^8$ and $(2.06\pm0.55)\times10^7$, respectively. For bacteria this indicator did not significantly differ from that in other sites, $(2.73\pm0.67)\times10^8$ for site No. 1 and $(2.55\pm0.37)\times10^8$ for site No. 3, whereas for archaea it was significantly ($p < 0.05$) higher than in two other sites, $(0.9\pm0.27)\times10^7$ in site No. 1 and $(1.52\pm0.39)\times10^7$ in site No. 3. The fungal abundance in the soil of different sites did not statistically significantly differ, $(1.88\pm0.26)\times10^7$ for No. 1, $(1.8\pm0.27)\times10^7$ for No. 2, and $(1.6\pm0.17)\times10^7$ for No. 3.

3. Diversity of soil microbiomes in various phytocenoses at sites of long-term restoration (M±SEM, quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016)

Site No.	Shannon index H	Faith's PD index	Chao 1	OTU number	Library coverage, %
1	9.3±0.1	171.1±9.7	2559.5±181.9	2163.8±165.5	84.5
2	9.4±0.1	169.6±10.5	2368.8±117.8	1978.8±176.6	83.5
3	9.3±0.1	188.3±10.1	2672.3±208.7	2322.3±176.8	86.9

N o t e. OTU — operational taxonomic unit; n = 4 for sites Nos. 1 and 2, n = 3 for site No. 3. Для участков №№ 1 и 2 n = 4, для участка № 3 n = 3. For description of sites by type of reclamation, see the section Materials and methods.

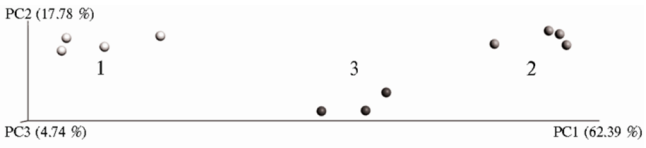

Fig. 3. Comparison of β-diversity of soil metagenomes with PCoA (Principal Components Analysis of the matrix of pairwise comparisons constructed by the weighted_unifrac method) **in various phytocenoses at sites of long-term restoration:** 1, 2, 3 — sites Nos. 1, 2 and 3 (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016). Axes are the projections of multidimensional data with the indicated values of the explained variation (%), the number of circles corresponds to the number of repetitions. For description of sites by type of reclamation, see the section Materials and methods.

For analysis of the soil microbiome of each site, DNA was isolated in 4 replicates. However, after sequencing for site No. 3 (pine), only 3 replicates were processed. Further, all measurements for sites No. 1 and No. 2 were in 4 replicates, and for section No. 3 in 3 replicates.

The indices of α-diversity and the number of microbiomes in all sites were comparable (Table 3). Figure 3 shows a β-diversity graph representing a three-dimensional projection of data from the matrix of pairwise distances between the microbiomes of soil samples constructed by weighted_unifrac method. It is seen that microbiomes clearly differentiate by the site of soil sampling.

Analysis of the diversity of the dominant bacterial phyla, *Proteobacetria*, *Actinobacteria*, and *Acidobacteria*, several patterns appeared (Table 4). Actinobacteria were the most diverse in microbiomes formed under dead cover in spruce forest (site No. 1), and the number of OTUs of this group of bacteria significantly decreased as spruce (site No. 1) > pine (site No. 3) > larch (site No. 2).

4. α-Diversity among bacterial groups of soil microbiomes in various phytocenoses at sites of long-term restoration (*M*±SEM, quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016)

Site No.	Shannon index H	Faith's PD index	Chao 1	OTU number
		Actinobacteria		
1	7.2±0.1[a]	23.9±0.5[a]	558.2±8.0[a]	311.9±5.1[a]
2	6.9±0.1[b]	21.9±0.4[a]	379.5±23.4[a]	272.1±5.8[a]
3	6.8±0.1[a]	23.1±0.7[b]	454.0±10.4[a]	291.5±6.4[b]
		Proteobacteria		
1	7.2±0.1[a]	43.5±1.3[ab]	702.0±37.6[a]	528.9±18.2[a]
2	7.7±0.1[a]	48.1±0.8[a]	742.6±31.7[b]	564.5±14.5[b]
3	7.3±0.2[b]	49.7±1.0[b]	850.6±44.3[c]	595.1±14.3[a]
		Acidobacteria		
1	6.4±0.1[a]	18.6±0.3[a]	343.7±16.9[a]	275.7±5.6[a]
2	6.0±0.3[b]	19.3±0.2[b]	389.0±16.8[b]	275.9±5.3[b]
3	6.3±0.4[c]	20.0±0.2[a]	455.3±21.4[a]	293.7±15.0[c]

N o t e. OTU — operational taxonomic unit; $n = 4$ for sites Nos. 1 and 2, $n = 3$ for site No. 3. For description of sites by type of reclamation, see the section Materials and methods. In the columns within the taxonomic group, the same letters (a, b, c) indicate statistically significant difference ($p < 0.05$).

Diversity of proteobacteria, on the contrary, was the highest in larch plantings (site No. 2) and the lowest in the spruce phytocenosis (site No. 1). It is also worth noting the reliable ($p < 0.05$) minimum phylogenetic diversity of proteobacteria (Faith's index) in the microbiome of the spruce forest, the reliable maximum ($p < 0.05$) of the number of detected OTUs under planted pine trees (site No. 3) and the maximum Shannon index for site No. 2 (larch) compared to site No. 1. The latter may indicate the most even appearance of various proteobacteria in the phytocenosis under the pine canopy. For members of *Acidobacteria* phylum, the general trend of increasing diversity and abundance under pine also remained (site No. 3). There were significant ($p < 0.05$) maxima of the phylogenetic diversity of *Acidobacteria* (Faith's index and Chao 1 index), characterizing the maximum richness of this group in site No. 3 compared to

site No. 1.

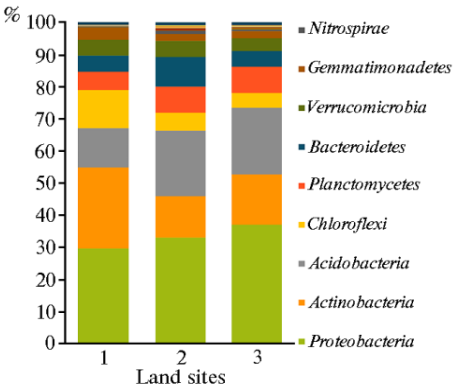

Fig. 4. Taxonomic profile of soil microbiome (procariote phyla) in various phytocenoses at sites of long-term restoration (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016). For each site, replicates are combined. Phyla representing > 1% are indicated. For description of sites by type of reclamation, see the section Materials and methods.

Analysis of the normalized 16S rRNA gene amplicon libraries for bacteria and archaea identified 220,910 nucleotide sequences. The dominant phyla were *Proteobacteria* (29.6-37%), *Actinobacteria* (12.8-25.2%) and *Acidobacteria* (12.3-20.8%), followed by members of phyla *Chloroflexi* (4.6-11.8%), *Planctomycetes* (5.7-8.2 %), *Bacteroidetes* (4.9-9.2%), *Verrucomicrobia* (3.9-5.0%), *Gemmatimonadetes* (2.1-3.8%), *Nitrospirae* (0.2-1.0%); the rest made less than 1% (Fig. 4). It is worth noting the relatively low representation (0.1%) and low diversity of archaea from the groups *Crenarchaeota* and [*Parvarchaeota*]. In general, the quantitative composition of phyla in different sites was similar, but it should be mentioned that in site No. 1 the abundance of *Actinobacteria* and *Chloroflexi* groups was higher compared to others, and the *Acidobacteria* were lower.

5. Heatmap of the major OUT percentage in soil microbiomes in various phytocenoses at sites of long-term restoration (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016)

Site No.			OTU	Phylum	Class	Order	Family	Genus
1	2	3						
			52697	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Bradyrhizobiaceae*	–
			9881*	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Rhodoplanes*
			39913	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Rhodoplanes*
			39246	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Rhodoplanes*
			36288*	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	–
			2794	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Sphingomonas*
			54700	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	–
			44729	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	–
			9787	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Delftia*
			18503*	*Proteobacteria*	*Betaproteobacteria*	IS-44	–	–
			18585	*Proteobacteria*	*Betaproteobacteria*	SC-I-84	–	–
			7276*	*Proteobacteria*	*Betaproteobacteria*	SC-I-84	–	–
			29307	*Proteobacteria*	*Deltaproteobacteria*	*Syntrophobacterales*	*Syntrophobacteraceae*	–
			33418	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*
			15473*	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Sinobacteraceae*	–
			35545	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	–
			46306	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	*Agromyces*
			18325*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	–
			32722*	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Promicromonosporaceae*	*Promicromonospora*
			37122*	*Actinobacteria*	MB-A2-108	0319-7L14	–	–
			45581	*Actinobacteria*	*Thermoleophilia*	*Gaiellales*	*Gaiellaceae*	–
			6537*	*Actinobacteria*	*Thermoleophilia*	*Gaiellales*	*Gaiellaceae*	–
			36062*	*Actinobacteria*	*Thermoleophilia*	*Solirubrobacterales*	–	–
			17100	*Actinobacteria*	*Thermoleophilia*	*Solirubrobacterales*	*Solirubrobacteraceae*	–
			6252*	*Acidobacteria*	*[Chloracidobacteria]*	RB41	–	–
			9076*	*Acidobacteria*	*[Chloracidobacteria]*	RB41	–	–
			20749*	*Acidobacteria*	*[Chloracidobacteria]*	RB41	Ellin6075	–
			22604*	*Acidobacteria*	*[Chloracidobacteria]*	RB41	Ellin6075	–
			54563	*Acidobacteria*	*Acidobacteria*-6	iii1-15	–	–
			52756	*Acidobacteria*	*Acidobacteria*-6	iii1-15	–	–

Continued Table 5

37100	*Acidobacteria*	*Acidobacteria*-6	iii1-15	–	–
43517	*Acidobacteria*	*Acidobacteria*-6	iii1-15	mb2424	–
4897	*Chloroflexi*	*Chloroflexi*	*[Roseiflexales]*	*[Kouleothrixaceae]*	–
32350	*Chloroflexi*	*Chloroflexi*	*[Roseiflexales]*	*[Kouleothrixaceae]*	–
57060*	*Chloroflexi*	*Chloroflexi*	*[Roseiflexales]*	*[Kouleothrixaceae]*	–
23485	*Chloroflexi*	Ellin6529	–	–	–
46192*	*Chloroflexi*	Ellin6529	–	–	–
11945	*Bacteroidetes*	*[Saprospirae]*	*[Saprospirales]*	*Chitinophagaceae*	–
54731	*Bacteroidetes*	*[Saprospirae]*	*[Saprospirales]*	*Saprospiraceae*	–
49979	*Bacteroidetes*	*Cytophagia*	*Cytophagales*	*Cytophagaceae*	*Adhaeribacter*
9879	*Verrucomicrobia*	*[Spartobacteria]*	*[Chthoniobacterales]*	*[Chthoniobacteraceae]*	*Chthoniobacter*
24870	*Verrucomicrobia*	*[Spartobacteria]*	*[Chthoniobacterales]*	*[Chthoniobacteraceae]*	DA101
20531	*Gemmatimonadetes*	Gemm-1	–	–	–
27129*	*Gemmatimonadetes*	*Gemmatimonadetes*	*N1423WL*	–	–
28624	*Planctomycetes*	*Phycisphaerae*	*CCM11a*	–	–

N o t e. OTU — operational taxonomic unit. OTU with a representation of > 0.5% in at least one of the options is considered major. For each OTU, the phylum and the lowest permitted taxon are indicated. An asterisk (*) marks OTUs, which representations differ more than 5-fold between at least a pair of sites ($p < 0.05$). Dashes mean that OTU is not identified at the marked taxon level. For description of sites by type of reclamation, see the section Materials and methods.

In total, among all replicates of the 16S rRNA gene libraries for the three studied sites, 5760 OTUs were identified. Of these, only 45 OTUs accounted for more than 0.5% of all sequences for at least one library (Table 5). They covered all 9 of the most represented bacterial phyla. The OTU taxonomic positions were determined mainly up to the levels of genera and families, but in some cases only up to classes and orders. Most OTUs belonged to the phylum *Proteobacteria* associated with the rhizosphere. The genus *Pseudomonas* which prevailed in soils under the pine trees was the most numerous. OTUs of the *Rhizobiales* and *Sphingomonadales* orders, closely associated with plants in the soil, are also common. For one of the OTUs of the *Rhizobiaceae* family, the excess in the soil under spruces was 5-fold compared to pines. Representatives of *Sinobacteraceae* are 5 times more abundant under pines than under spruces. The genus *Rhodoplanes* is associated with the decomposition of wood in forest soils [45]. OTUs belonging to these orders were more often found in site No. 1, which was recultivated earlier than the others.

The genus *Delftia*, on the contrary, was more characteristic of younger replantozems in sites No. 2 and No. 3. Its appearance is associated with active bioremediation processes in soils [46, 47]. The second most numerous OTU group was the *Actinobacteria* phylum (classes *Actinobacteria*, *Thermoleophillia* and MB-A2-108). The most numerous OTUs belonged to the families *Micrococcaceae* and *Microbacteriaceae*, whose members, including the genus *Agromyces* of biodestructors [48]. Moreover, in the soils under spruces and larches *Micrococcaceae* significantly (10-fold) increased compared to the soil under pines. In site No. 1, the count of *Promicromonospora actinobacteria* was significantly higher (20 times, $p < 0.05$) than in sites No. 2 and No. 3. Among acidobacteria, the *Chloracidobacteria* class dominated in all sites; the second largest class was *Acidobacteria*-6. OTUs of the order RB41, dominant in sites No. 2 and No. 3, are often described in metagenomes of disturbed and stressed soils [49-51]. Among the minor phyla, OTUs of classes *Chloroflexi* and Ellin6529, which dominate in site No. 1, should be noted. Bacteria of Ellin6529 class can fix atmospheric nitrogen [52]. Also, in site No. 1, bacteria of the genus DA101 from *Verrucomicrobia* phylum prevailed.

In our study, we did not find any correlation between the soil microbiome composition and soil-forming processes, in particular, the decomposition of organic residues in the soil and their mineralization. The Mantel test with

weighted_unifrac-derived community distance matrices did not reveal a statistically significant correlation of the composition of the microbiome with pH or the main biogenic soil elements (C, N, P, and K) (Table 6).

6. Correlation of soil microbiome β-diversity with main chemical soil parameters in various phytocenoses at sites of long-term restoration (quarry No. 3 of PO Phosphorite, Kingisepp phosphorite deposit, Leningrad Province, Kingisepp District, 2016))

Parameter	*r*	p-value
pH	0.99064	0.1638
Concentration:		
C	–0.02333	1.0000
N_{total}	–0.67638	0.6840
N (NH_4)	0.76821	0.4973
N (NO_3)	0.48269	0.8311
P (P_2O_5)	0.08478	1.0000
K (K_2O)	0.51337	0.6560

N o t e. All replicates for all sites are combined. For description of sites by type of reclamation, see the section Materials and methods.

In the phosphate rock quarry in Kingisepp district, forest communities were formed that are typical of the taiga zone of the European Russia. The organomineral horizon of site No. 1 was more acidic which may be due to the introduction of a substrate with an admixture of peat at reclamation. As per the data on basal and substrate-induced respiration, the microbiological activity of soils is low, which may indicate a reduced sustainability of microbial communities.

In all site we revealed a rather low α-diversity. This may be a consequence of the low intensity of soil formation on crushed-stone dumps, which is in good agreement with the earlier data for podzolic and sod-podzolic soils of the Northwest region [53]. The indices of α-diversity were similar for microbiomes of all sites, while in site No. 3 (under the pine), a significant increase in the species and phylogenetic diversity of the microbial community was recorded. But despite the fact that for the sites the indices of α-diversity were generally comparable, their values significantly changed for the three dominant phyla in the soil microbiomes formed under different plant communities (see Table 4). So, for site No. 1, a relative increase in diversity, as well as the proportion of actinobacteria, was characteristic, while the diversity and abundance of proteobacteria was maximum in pine plantings (site No. 3). Actinobacteria and proteobacteria often turn out to be antagonists in a soil microbiome [54, 55]. An interesting fact is that the soil in site No. 1 had the lowest pH value, although actinobacteria in most cases inhabit soils of neutral or closer to alkaline pH [56]. In the sites, most likely, it is the composition of the litter and the availability of organic matter for microbial decomposition, which, apparently, differs due to the uneven application of peat in different areas, have a more decisive effect on the structure of the microbiome than pH. It is likely that the soil formed under the spruce canopy differed from soils of other sites in organic matter of low accessibility for the microbial community. This may explain the relative increase in the diversity and abundance of actinobacteria, most of which are hydrolytic with oligotrophic nutrition type [57]. In site No. 3, on the contrary, the diversity of higher plants and their life forms is the greatest. This suggests a wider spectrum and a wider variety of organic substances, including readily available ones, capable, in turn, to affect the microbiome, in particular, the copiotrophic microorganisms, to which the majority of *Proteobacteria* phylum members belongs [58].

Taxonomic analysis we performed for the reclaimed soils showed the formation of a microbial community characteristic of acidified sod-podzolic and

podzolic soils of the Northwest [53] with prevalence of acidobacteria of orders RB41, iii1-15, as well as actinobacteria (*Gaiellaceae* and *Solirubrobacteriaceae* families). The appearance of these groups, as well as a significant proportion of bacteria from the phyla *Bacteroidetes*, *Verrucomicrobia*, and *Planctomycetes* (their members are mainly oligotrophs) [59, 60], indicate the completeness of the carbon cycle and stabilization of the microbial community composition. However, the observed stage, apparently, should be considered as pre-climax, since in all analyzed communities there are many copiotrophic bacteria, in particular, pseudomonads (on average 5.5%) [61]. Noteworthy, proteobacteria (family *Comamonadaceae*, genus *Delftia*) are detected in all sites. In the literature, this microorganism is often found in communities of contaminated and technologically disturbed soils, as well as in activated sludge microbiomes [62]. Apparently, this bacterium takes part in the soil formation in the reclaimed sites under examination.

So, the study we undertook in the reclamation areas of Leningrad Province (Kingisepp phosphorite deposit) did not reveal a relationship between the structure of the soil microbiome and the physicochemical parameters of soils. In addition, the diversity of soil microbiome does not correlate with soil concentration of the main mineral elements. The factor of the plant dominant also does not have a significant effect on the microbiome structure. It is possible that in the case under discussion, other environmental factors are of decisive importance for microorganisms. The supposed factors may be different composition of plant waste and litter and, as a consequence, the diversity of organic substrates. Analysis of the α-diversity and taxonomic composition of microbiomes at three test sites suggests a pre-climax stage of the soil cover development. At the same time, the soil parameters and the high content of mineral phosphorus turn out to be too strong abiotic factors that impede the complete restoration of the microbiome of disturbed soils.

REFERENCES

1. Dmitrakova Ya.A., Abakumov E.V., Pershina E.A., Ivanova E.A., Andronov E.E. *Dynamics of the plant community and microbiom of chrono-series of post-technological soil in limestone quarry in the conditions of recultivation*. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(3): 557-569 (doi: 10.15389/agrobiology.2018.3.557eng).
2. Bing-yuan H., Li-xun K. Mine land reclamation and eco-reconstruction in Shanxi province I: mine land reclamation model. *The Scientific World Journal*, 2014, 2014: 483862 (doi: 10.1155/2014/483862).
3. Sumina O.I. *Formirovanie rastitel'nosti na tekhnogennykh mestoobitaniyakh Krainego Severa Rossii. Avtoreferat kandidatskoi dissertatsii* [Formation of vegetation in technogenic habitats of the Far North of Russia. PhD Thesis]. St. Petersburg, 2011 (in Russ.).
4. Abakumov E.V., Gagarina E.I. *Pochvoobrazovanie v posttekhnogennykh ekosistemakh kar'erov na Severo-Zapade Russkoi ravniny* [Soil formation in post-technogenic quarry ecosystems in the North-West of the Russian Plain]. St. Petersburg, 2006 (in Russ.).
5. Valdez-Hernández M., Gil-Medina R., Lypez-Martínez J.O., Torrescano-Valle N., Cabanillas-Terán N., Islebe G.A. Succession and the relationship between vegetation and soil in the Marl Quarries of the Yucatan Peninsula, Mexico. *Forests*, 2019(2), 10: 116 (doi: 10.3390/f10020116).
6. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, 2017, 15: 579-590 (doi: 10.1038/nrmicro.2017.87).
7. Sergaki C., Lagunas B., Lidbury I., Gifford M.L., Schäfer P. Challenges and approaches in microbiome research: from fundamental to applied. *Frontiers in Plant Science*, 2018, 9: 1205 (doi: 10.3389/fpls.2018.01205).
8. Torsvik V., Øvreas L. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*, 2002, 5(3): 240-245 (doi: 10.1016/s1369-5274(02)00324-7).
9. Wei Y.J., Wu Y., Yan Y.Z., Zou W., Xue J., Ma W.R., Wang W., Tian G., Wang L.Y. High-throughput sequencing of microbial community diversity in soil, grapes, leaves, grape juice and wine of grapevine from China. *PLoS One*, 2018, 13(3): e0193097 (doi: 10.1371/journal.pone.0193097).

10. Soliman T., Yang S.Y., Yamazaki T., Jenke-Kodama H. Profiling soil microbial communities with next-generation sequencing: the influence of DNA kit selection and technician technical expertise. *PeerJ*, 2017, 5: e4178 (doi: 10.7717/peerj.4178).
11. Janssen P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA Genes. *Applied and Environmental Microbiology*, 2006, 72(3): 1719-1728 (doi: 10.1128/AEM.72.3.1719).
12. Hua J., Feng Y., Jiang Q., Bao X., Yin Y. Shift of bacterial community structure along different coastal reclamation histories in Jiangsu, Eastern China. *Scientific Reports*, 2017, 7(1): 10096 (doi: 10.1038/s41598-017-10608-3).
13. Lauber C.L., Hamady M., Knight R., Fierer N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 2009, 75(15): 5111-5120 (doi: 10.1128/AEM.00335-09).
14. Karimi B., Terrat S., Dequiedt S., Saby N.P.A., Horrigue W., Lelièvre M., Nowak V., Jolivet C., Arrouays D., Wincker P., Cruaud C., Bispo A., Maron P.-A., Bouré N.C.P., Ranjard L. Biogeography of soil bacteria and archaea across France. *Science Advances*, 2018, 4(7): eaat1808 (doi: 10.1126/sciadv.aat1808).
15. Valencia E., Gross N., Quero J.L., Carmona C.P., Ochoa V., Gozalo B., Delgado - Baquerizo M., Dumack K., Hamonts K., Singh B.K., Bonkowski M., Maestre F.T. Cascading effects from plants to soil microorganisms explain how plant species richness and simulated climate change affect soil multifunctionality. *Global Change Biology*, 2018, 24(12): 5642-5654 (doi: 10.1111/gcb.14440).
16. Hu L., Robert C.A.M., Cadot S., Zhang X., Ye M., Li B., Manzo D., Chervet N., Steinger T., van der Heijden M.G.A., Schlaeppi K., Erb M. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, 2018, 9(1): 2738 (doi: 10.1038/s41467-018-05122-7).
17. Hartman K., van der Heijden M.G.A., Wittwer R.A., Banerjee S., Walser J.C., Schlaeppi K. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome*, 2018, 6(1): 14 (doi: 10.1186/s40168-017-0389-9).
18. Pershina E.V., Ivanova E.A., Korvigo I.O., Chirak E.L., Sergaliev N.H., Abakumov E.V., Provorov N.A., Andronov E.E. Investigation of the core microbiome in main soil types from the East European plain. *Science of the Total Environment*, 2018, 631-632: 1421-1430 (doi: 10.1016/j.scitotenv.2018.03.136).
19. Thavamani P., Samkumar R.A., Satheesh V., Subashchandrabose S.R., Ramadass K., Naidu R., Venkateswarlu K., Megharaj M. Microbes from mined sites: harnessing their potential for reclamation of derelict mine sites. *Environmental Pollution*, 2017, 230: 495-505 (doi: 10.1016/j.envpol.2017.06.056).
20. Abakumov E.V., Gagarina E.I., Lisitsyna O.V. Land reclamation in the Kingisepp area of phosphorite mining. *Eurasian Soil Science*. 2005, 38(6): 648-655.
21. Rastvorova O.G. *Fizika pochv — prakticheskoe rukovodstvo* [Soil physics — a practical guide]. Leningrad, 1983 (in Russ.).
22. Rastvorova O.G., Andreev D.P., Gagarina E.I., Kasatkina G.A., Fedorova N.N. *Khimicheskii analiz pochv* [Chemical analysis of soils]. St. Petersburg, 1995 (in Russ.).
23. *GOST R 54650-2011. Pochvy. Opredelenie podvizhnykh soedinenii fosfora i kaliya po metodu Kirsanova v modifikatsii TSINAO* [GOST R 54650-2011. The soil. Determination of mobile compounds of phosphorus and potassium according to the Kirsanov method in the modification of TsINAO]. Moscow, 2011 (in Russ.).
24. *GOST 26489-85. Pochvy. Opredelenie obmennogo ammoniya po metodu TSINAO* [GOST 26489-85. The soil. Determination of exchange ammonium by the method of TsINAO]. Moscow, 1985 (in Russ.).
25. *GOST 26951-86. Pochvy. Opredelenie nitratov ionometricheskim metodom* [GOST 26951-86. The soil. Determination of nitrates by the ionometric method]. Moscow, 1986 (in Russ.).
26. Anan'eva N.D., Sus'yan E.A., Gavrilenko E.G. *Pochvovedenie*, 2011, 11: 1327-1333 (in Russ.).
27. Andronov E.E., Petrova S.N., Chizhevskaya E.P., Korostik E.V., Akhtemova G.A., Pinaev A.G. *Mikrobiologiya*, 2009, 78(4): 525-534 (in Russ.).
28. Lane D.J. 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematic*. E. Stackebrandt, M. Goodfellow (eds.). John Wiley and Sons, New York, 1991: 115-175.
29. Muyzer G., de Waal E.C., Uitterlinden A.G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 1993, 59(3): 695-700.
30. Yu Y., Lee C., Hwang, S. Analysis of community structures in anaerobic processes using quantitative real-time PCR method. *Water Science and Technology*, 2005, 52(1-2): 85-91 (doi: 10.2166/wst.2005.0502).
31. Gardes M., Bruns T.D. ITS primers with enhanced specificity for basidiomycetes — application

to the identification of mycorrhizae and rusts. *Molecular Ecology*, 1993, 2(2): 113-118 (doi: 10.1111/j.1365-294X.1993.tb00005.x).
32. Bates S.T., Berg-Lyons D., Caporaso J.G., Walters W.A., Knight R., Fierer N. Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal*, 2011, 5(5): 908-917 (doi: 10.1038/ismej.2010.171).
33. Bolger A.M., Lohse M., Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 2014, 30(15): 2114-20 (doi: 10.1093/bioinformatics/btu170).
34. Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peca A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 2010, 7: 335-336 (doi: 10.1038/nmeth.f.303).
35. Edgar R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 2010, 26(19): 2460-2461 (doi: 10.1093/bioinformatics/btq461).
36. Caporaso J.G., Bittinger K., Bushman F.D., DeSantis T.Z., Andersen G.L., Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*, 2009, 26(2): 266-267 (doi: 10.1093/bioinformatics/btp636).
37. DeSantis T.Z., Hugenholtz P., Larsen N., Rojas M., Brodie E.L., Keller K., Huber T., Dalevi D., Hu P., Andersen G.L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, 2006, 72(7): 5069-5072 (doi: 10.1128/AEM.03006-05).
38. Chao A. Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 1984, 11: 265-270.
39. Shannon C.E., Weaver W. *The mathematical theory of communication*. Urbana, University of Illinois Press, 1949.
40. Faith D.P. Conservation evaluation and phylogenetic diversity. *Biological Conservation*, 1992, 61(1): 1-10 (doi: 10.1016/0006-3207(92)91201-3).
41. Lozupone C., Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, 2005, 71(12): 8228-8235 (doi: 10.1128/AEM.71.12.8228-8235.2005).
42. Vázquez-Baeza Y., Pirrung M., Gonzalez A., Knight R. EMPeror: a tool for visualizing high-throughput microbial community data. *GigaScience*, 2013, 2(1): 16 (doi: 10.1186/2047-217X-2-16).
43. Mantel N. The detection of disease clustering and a generalized regression approach. *Cancer Research*, 1967, 27(2 Part 1): 209-220.
44. Mann H.B., Whitney D.R. On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics*, 1947, 18(1): 50-60.
45. Tláskal V., Zrůstová P., Vrška T., Baldrian P. Bacteria associated with decomposing dead wood in a natural temperate forest. *FEMS Microbiology Ecology*, 2017, 93(12): fix157 (doi: 10.1093/femsec/fix157).
46. Liu J., Xu G., Dong W., Xu N., Xin F., Ma J., Fang Y., Zhou J., Jiang M. Biodegradation of diethyl terephthalate and polyethylene terephthalate by a novel identified degrader *Delftia* sp. WL-3 and its proposed metabolic pathway. *Letters in Applied Microbiology*, 2018, 67(3): 254-261 (doi: 10.1111/lam.13014).
47. Liu Y., Tie B., Li Y., Lei M., Wei X., Liu X., Du H. Inoculation of soil with cadmium-resistant bacterium Delftia sp. B9 reduces cadmium accumulation in rice (*Oryza sativa* L.) grains. *Ecotoxicology and Environmental Safety*, 2018, 163: 223-229 (doi: 10.1016/j.ecoenv.2018.07.081).
48. Wang R., Zhang H., Sun L., Qi G., Chen S., Zhao X. Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. *Scientific Reports*, 2017, 7: 343 (doi: 10.1038/s41598-017-00472-6).
49. Awasthi M.K., Li J., Kumar S., Awasthi S.K., Wang Q., Chen H., Wang M., Ren X., Zhang Z. Effects of biochar amendment on bacterial and fungal diversity for co-composting of gelatin industry sludge mixed with organic fraction of municipal solid waste. *Bioresource Technology*, 2017, 246: 214-223 (doi: 10.1016/j.biortech.2017.07.068).
50. Shen Y., Ji Y., Li C., Luo P., Wang W., Zhang Y., Nover D. Effects of phytoremediation treatment on bacterial community structure and diversity in different petroleum-contaminated soils. *International Journal of Environmental Research and Public Health,* 2018, 15(10): 2168 (doi: 10.3390/ijerph15102168).
51. Jurburg S.D., Natal-da-Luz T., Raimundo J., Morais P.V., Sousa P., van Elsas J.D., Salles J.F. Bacterial communities in soil become sensitive to drought under intensive grazing. *Science of The Total Environment*, 2018, 618: 1638-1646 (doi: 10.1016/j.scitotenv.2017.10.012).
52. Lopes A.R., Bello D., Prieto-Fernández Á., Trasar-Cepeda C., Manaia C.M., Nunes O.C. Relationships among bulk soil physicochemical, biochemical, and microbiological parameters in

an organic alfalfa-rice rotation system. *Environmental Science and Pollution Research*, 2015, 22(15): 11690-11699 (doi: 10.1007/s11356-015-4410-1).
53. Chirak E.L., Pershina E.V., Dol'nik A.S., Kutovaya O.V., Vasilenko E.S., Kogut B.M., Merzlyakova Ya.V., Andronov E.E. Taxonomic structure of microbial association in different soils investigated by high-throughput sequencing of 16S-rRNA gene library. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2013, 3: 100-109 (doi: 10.15389/agrobiology.2013.3.100eng).
54. Ren C., Sun P., Kang D., Zhao F., Feng Y., Ren G., Han X., Yang G. Responsiveness of soil nitrogen fractions and bacterial communities to afforestation in the Loess Hilly Region (LHR) of China. *Scientific Reports*, 2016, 6: 28469 (doi: 10.1038/srep28469).
55. Wu B., Hou S., Peng D., Wang Y., Wang C., Xu F., Xu H. Response of soil micro-ecology to different levels of cadmium in alkaline soil. *Ecotoxicology and Environmental Safety*, 2018, 166: 116-122 (doi: 10.1016/j.ecoenv.2018.09.076).
56. Yun Y., Wang H., Man B., Xiang X., Zhou J., Qiu X., Duan Y., Engel A.S. The relationship between pH and bacterial communities in a single karst ecosystem and its implication for soil acidification. *Frontiers in Microbiology*, 2016, 7: 1955 (doi: 10.3389/fmicb.2016.01955).
57. Arocha-Garza H.F., Canales-Del Castillo R., Eguiarte L.E., Souza V., De la Torre-Zavala S. High diversity and suggested endemicity of culturable Actinobacteria in an extremely oligotrophic desert oasis. *Journal of Life and Environmental Sciences*, 2017, 5: e3247 (doi: 10.7717/peerj.3247).
58. Rosenfeld C.E., James B.R., Santelli C.M. Persistent bacterial and fungal community shifts exhibited in selenium-contaminated reclaimed mine soils. *Applied and Environmental Microbiology*, 2018, 84(16): e01394-18 (doi: 10.1128/AEM.01394-18).
59. Leff J.W., Fierer N. Bacterial communities associated with the surfaces of fresh fruits and vegetables. *PLoS ONE*, 2013, 8(3): e59310 (doi: 10.1371/journal.pone.0059310).
60. Masse J., Prescott C.E., Renault S., Terrat Y., Grayston S.J. Plant community and nitrogen deposition as drivers of alpha and beta diversities of prokaryotes in reconstructed oil sand soils and natural boreal forest soils. *Applied and Environmental Microbiology*, 2017, 83(9): e03319-16 (doi: 10.1128/AEM.03319-16).
61. Lladó S., Baldrian P. Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *PLoS ONE*, 2017, 12(2): e0171638 (doi: 10.1371/journal.pone.0171638).
62. Hou Q., Wang C., Guo H., Xia Z., Ye J., Liu K., Yang Y., Hou X., Liu H., Wang J., Du B., Ding Y. Draft genome sequence of *Delftia tsuruhatensis* MTQ3, a strain of plant growth-promoting Rhizobacterium with Antimicrobial Activity. *Genome Announcements*, 2015, 3(4): e00822-15 (doi: 10.1128/genomeA.00822-15).