Plant and soil

DYNAMICS OF THE PLANT COMMUNITY AND MICROBIOM OF CHRONO-SERIES OF POST-TECHNOLOGICAL SOIL IN LIMESTONE QUARRY IN THE CONDITIONS OF RECULTIVATION

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A b s t r a c t

Post-technogenic ecosystems represent informative natural models of initial pedogenesis and restoration under the abandoned and reclamation practices. These soils can be considered as natural experiments on restoration of microbial communities within the age row (time series or chronoseries). Investigation of different aged stages of soil formation on the spoil banks of the quarries give a possibility to obtain initial data on the rate and trends of the pedogenesis in various combinations of substrates and phytocenosis. Among the quarries of mineral substrates in North-West region, particular place is devoted to quarries for lime stone exploitation located on Izhora upland. The purpose of this study conducted in one of the largest limestone quarries of Leningrad region was to examine succession of plant and microbial communities. Species composition and vegetation cover were estimated for different plant communities within each ecotype of quarry. Also at the each plot the following characteristics of soil were measured: pH; organic carbon; soil basal respiration and substrate induced respiration; texture; CO_2 of carbonates and moisture content. Total soil DNA was exctracted using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., USA), sequencing of v4, the variable region of 16S rRNA gene pDNA, was conducted using a third-generation GS Junior sequenator (Roshe, Switzerland). The results were processed using the QIIME software. To compare microbial communities, the alpha and beta diversity analyses were performed. Our results highlighted that the main difference between plant communities of different plots were due to position in the landscape, most similar communities colonize similar ecotypes. The microbial communities of the old (35 years or more) dumps are essentially specific, and communities of microorganisms of young (8-16 years) and middle-aged (28-30 years) dumps tend to group into separate clusters. As compared to young and middle age dumps, old dumps are characterized by a significant (6-8-fold) increase in the counts of *Micromonosporaceae* and *Sinobacteraceae* representatives. Communities of young dumps have 4.2 times more *Pseudomonas* and 3.8 times more *Micrococcaceae* members than the communities of old ones. The communities of wet terraces also differ from the microbiomes of the other ecotopes (dry terraces, the bases of the dumps), however, no significant differences could be identified. The main issue for successful soil reclamation is the restoration of the microbial community. It was shown that copiotrophes, the microorganisms adapted to high concentration of soil nutrients, dominate in the youngest soils. As microbial succession proceeded, oligotrophs which are involved in organic matter decomposition become dominating. It was established that the processes connected with transformation of organic matter became the main drivers of soil formation, which is especially important for initial stages of soil body restoration. Data on soil microbiomes and microbiomes of soil-vegetation complexes could be the most important tools for reclamation practices.

Keywords: primary succession of plant and microbial communities, limestone quarries, pedogenesis, metagenome, reclamation

The increase in the territories of disturbed lands, occurring as a result of the development and extraction of minerals, is a particular environmental problem. As a rule, anthropogenic landscapes are removed from the forest and agriculturally used areas, and the processes of soil and vegetation cover remediation are extremely slow [1]. The study of soil formation under impact of technogenesis is a theoretical basis for reclamation of disturbed lands and recovery of landscapes [2, 3]. Rendzina soils formed on the Izhora Plateau are characterized by high fertility and good drainage conditions, so these territories have been actively used in agriculture for a long time [4]. The high carbonate content in the dump rock results in the potential fertility of calcareous dumps, but the specificity of the composition of quarry-dumping complexes, as well as a relatively low content of nutrients and unfavorable physical properties of the substrate, creates difficulties in reclamation of these territories [5]. When selecting approach for reclamation, leveling the surface becomes quite a laborious and expensive task [3]. However, the complex terrain formed during the development of quarries provides a high variety of ecological niches, which are favorable for a large number of plant and animal species, including those valuable for natural conservation. Thus, in various conditions and geographical zones, it was found that abandoned quarries can increase biodiversity by acting as refugiums for rare species of plants and animals [6, 7].

The world practice of reclamation emphasizes the use of regeneration capabilities of natural ecosystems, where the biotic factors of the soil-forming process, the vegetation cover and associated soil microbiome play a critical role [8, 9]. The activity of microorganisms is directly related to the fertility and the provision of soil ecosystem functions, which include the balance of the nutrient elements cycle, as well as the ecological adaptation of plants to stressful conditions [10]. In addition, the high adaptive potential of soil microorganisms that are capable of adapting to any environmental changes in a short time and to use all available ecological niches allows the use of the microbiome structure as one of the most sensitive environmental indicators that mark different stages of soil remediation [11].

The spontaneous succession of plant communities in quarries with various substrates has been studied in sufficient detail. A significant part of domestic studies is related to various aspects of the succession vegetation cover [12-14], and the processes of pedogenesis have been analyzed sufficiently [15, 16]. However, complex observations on remediation of the soil and vegetation cover and the connection of these components remain insufficient. The majority of foreign papers, on the contrary, are focused on the conjugate development of the soil and vegetation cover after planting certain plant species, but not due to self-organized vegetation [17-20]. Traditionally, papers on the soil reclamation are focused on the study of vegetation cover and redistribution of soil organic matter [9, 15, 16]. The importance of a microbiome in ecosystem restoration processes has been underestimated for a long time. Over the recent decade, many publications [21-23] have been focused on the study of the microbiome of reclaimed soils and contain valuable information on the rates of biomass restoration and the activity of microbial communities in the chronoseries of soils in technogenic landscapes [24-26]. It has been shown that the structure and composition of microbiomes are determined by a combination of physical and agrochemical parameters (reserves of soil organic matter, total nitrogen, pH, cation exchange capacity), the values of which are significantly higher in reclaimed variants. Much attention is paid to the use of microbial communities as indicators of different stages of technogenic landscapes soil remediation [27-29]. At the same time, the diversity and structure of microbiomes in disturbed and reclaimed soils is still insufficiently studied. Only with the advent of metagenomic technologies, it became possible to effectively analyze and interpret the diversity of soil microbiota. Involvement in the study

on the soil microbiome diversity creates opportunities for the development of qualitatively new systems for accelerating and optimizing reclamation measures in disturbed areas. Recently, attention has been paid to the creation of microbial preparations to improve the quality of soils [30, 31].

A comprehensive study of the three main components of the ecosystem (soil, plant community and microbiome) in chronoseries of various ecotopes in conditions of reclamation and self-organized vegetation was carried out in the territory of one of the largest limestone quarries in the Leningrad Region for the first time. The taxonomic structure of the microbiome of the initial soils has been established both for quarries located in the Northwest and for the initial soils of Russia as a whole.

The purpose of the paper was to study the dynamics of phytocoenosis and soil microbiome in the process of succession on dumps of different ages at natural self-organized vegetation and reclamation of the limestone quarry.

Techniques. The study was carried out on a limestone quarry Pechurki (Slantsevsky District, Leningrad Region) during the spring and summer of 2016. Limestone mining was stopped here in 2014. First of all, the main ecotopes of the quarry, corresponding to the elementary soil areal, were determined. 12 test sites 25×25 m in size were set within each ecotope. The total plant cover and species composition of higher vascular plants, ground mosses and lichens, were evaluated on sites. Soil cuts were also set within each plot. Field descriptions of the soils were carried out on each of the open test pits, samples (700 g each) were taken from the pedogenic horizons to perform laboratory analyzes.

The following indicators were determined: substrate-induced respiration (SIR) of soil samples [32]; basal respiration according to the procedure for SIR [32], but in soil not enriched with substrate; the content of organic carbon in bichromate oxidation (I.V. Tyurin's method); pH of the aqueous suspension and saline pH (1:2.5 soil:solution); exchange and hydrolytic acidity; CO_2 carbonates content using the acidimetric method [33]; hygroscopic and maximum hygroscopic humidity, as well as total water capacity (moisture storage capacity) and the lowest water capacity using gravimetry method [34]; the density of soil and solid phase of soils, the structure and rockiness of soil using the method of dry screening; the grain-size composition using Kachinsky's pipette method with phosphate peptization of microaggregates [34]; the fraction-group composition of humus according to the scheme of I.V. Tyurin, modified by V.V. Ponomareva et al. [35].

The amount of carbon of the microbial biomass was calculated according to the formula of J.P.E. Anderson et al. [36]: C_{mic} (μg C/g soil) = SIR (μl CO_2/g soil $\cdot$ h^{-1}) × 40.04 + 0.37, where SIR is substrate-induced respiration. The microbial metabolic coefficient (specific respiration of microbial biomass) was determined as the ratio of basal respiration (BR) to the indicator C_{mic}: qCO_2 (μg CO_2-C/mg $C_{mic} \cdot$ h^{-1}) = BR/C_{mic}.

The similarity coefficient (the Sørensen-Czekanowski coefficient) was calculated according to the lists of higher plants species prepared for each site: K = 2c/(a + b), where a is the number of species at the first site, b is the number of species at the second site, c is the number of common species for the first and second sites. The most typical coefficients of biodiversity were also determined for each site. The Simpson index (C) was calculated by the formula: C = 1 − $\sum(n_i/N)^2$, where n_i is an estimate of each species significance (projective cover), N is the sum of significance estimates. The Shannon index was calculated according to the formula: H = −$\sum n_i/N$ × log (n_i/N).

The method of forward selection with two limiting criteria [37] for reducing the type I error was used to evaluate the effect of environmental factors on the phytocenosis structure. The canonical correspondence analysis (CCA)

proposed by C.J.F. Ter Braak [38] was performed using only significant variables (p < 0.05) in order to determine the effect of environmental factors on the distribution of plant communities.

Soil samples were taken from a depth of 8-10 cm at sites No. 1-9. DNA was isolated from the samples (0.2 g) using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., USA) in accordance with the manufacturer's instructions. Purified DNA preparations (10-15 ng) were matrices in polymerase chain reaction (PCR) (temperature profile: 30 s at 95 °C, 30 s at 50 °C, 30 s at 72 °C; total 30 cycles) using En-cyclo DNA polymerase (Eurogen, Russia) and universal primers at the variable region v4 of the 16S rRNA gene: F515 (GTGCCAG-CMGCCGCGGTAA) and R806 (GGACTACVSGGGTATCTAAT). The oligonucleotide identifiers were introduced into the primers for each sample and the service sequences necessary for the pyrosequencing according to the Roche protocol (Switzerland). Samples preparation and sequencing were performed using GS Junior (Roche, Switzerland) in accordance with the manufacturer's recommendations. Demultiplexing, quality control of 16S rRNA gene sequences, isolation of OTU (operational taxonomic units, species analog), normalization of samples, taxonomic identification of OTU, calculation of α- and β-diversity indices were performed with QIIME software package (http://qiime.org/) using the default parameters [39, 40]. Libraries of 16S rRNA gene fragments were standardized according to the number of sequences in the smallest library.

Differences in the frequencies of microbial taxa between the test samples were determined by an accurate Fisher test, adjusted for multiple comparisons using the Benjamini-Hochberg procedure at a 5% significance level. In order to explain the variability in the composition of species of microbial communities under the influence of the environmental factor, the Principal Component Analysis (PCoA) was used. Calculations were carried out with Statistica 7 software (StatSoft, Inc., USA) and Microsoft Excel.

Results. Works on reclamation in the limestone quarry Pechurki began in 1970, during which pine seedlings were planted in some areas at different times. In this case, most of the quarry was left for self-organized vegetation. The quarry was characterized by a complex internal relief, which contributed to the formation of a relatively wide variety of soils. Most environmental factors were optimal for the development of vegetation (Table), except for high density and rockiness on the rocky bottom of the quarry. All sites showed an extremely heterogeneous distribution of fractions along the section and a significant amount of skeletal coarse clastic material with a relatively low content of fine soil. In general, the results of mesomorphological studies indicated a fairly high rate of pedogenic transformation of the substrate. Chemical, biochemical and physical weathering of carbonate rocks are the basic transformation processes of the mineral part of the soil. The high content of fine earth contributed to the intensive weathering of limestone fragments (except for the rocky bottom of the quarry). This, in turn, increased the water capacity of embryozem and its fertility, thereby ensuring the active development of plant communities that produce organic matter. Intensive decarbonization was in a significant decrease in the pH of the humus horizons in comparison with the rock.

Places for establishment of test sites were selected in such a manner as to avoid the border effect (the density and number of species increases at the joints of biocenoses which can lead to distorted results), that is, preferably in the central part of the phytocenosis (if available) or in the central part of the ecotope (in the absence of closed vegetation cover). The location of the test sites is shown in Figure 1. The key pedogenic processes in all areas were the accumulation of organic matter and the substrate oxidation. They led to the formation of an A-horizon with maximum power and the largest content of organic matter in

Characterization of soils formed in the limestone quarry Pechurki (Slantsevsky District, Leningrad Region, 2016)

A	B	C	D	E	F	G	H	I	J	K	L
1	O	0-4	5.02	0.00	8.16	0.85	0.07	0.07	3.225	0.021	13.1
	AY	4-33	4.40	0.06	6.79	—	0.03	0.03	1.695	0.018	
	C	33-48	5.72	0.10	1.19	—	0.02	0.02	1.186	0.017	
	[C]	48+	7.45	0.17	0.86	—	0.03	0.03	1.491	0.017	
2	O	0-3	5.26	0.00	1.69	0.76	0.11	0.12	5.060	0.022	30.5
	BF	3-13	6.35	0.24	5.73	—	0.04	0.04	2.103	0.019	
	C	13+	6.55	0.08	2.05	—	0.04	0.04	1.797	0.020	
3	O	0-7	6.36	0.10	3.27	0.78	0.04	0.05	2.205	0.018	6.9
	AY	7-15	5.86	0.10	7.85	—	0.02	0.03	1.491	0.015	
	G	15-36	6.43	0.07	0.65	—	0.06	0.07	3.021	0.020	
	G_{ox}	36-45	6.12	0.08	1.94	—	0.04	0.04	2.001	0.019	
	C	45	6.50	0.09	5.45	—	0.02	0.02	0.982	0.016	
4	AY	0-26	5.22	0.21	3.94	0.91	0.04	0.05	2.205	0.018	29.5
	AC	26+	6.34	0.16	6.32	—	0.02	0.02	0.982	0.016	
5	O	0-13	5.64	0.00	14.4	0.65	0.04	0.05	2.205	0.020	28.0
	AY	13-25	6.05	0.35	2.13	—	0.06	0.07	3.021	0.021	
	C1	25-37	5.76	0.08	0.67	—	0.02	0.03	1.390	0.018	
	$C2_{ox}$	37+	6.10	0.52	3.33	—	0.03	0.04	1.797	0.017	
6	O	0-4	6.57	0.00	24.81	—	0.06	0.08	2.817	0.022	13.8
	AY	4-28	6.53	0.13	13.25	0.76	0.04	0.04	2.001	0.018	
	C	28+	5.40	0.27	4.47	—	0.04	0.04	1.797	0.020	
7	O	0-7	5.98	0.00	8.24	—	0.07	0.08	3.429	0.019	4.5
	AY	7-9	6.90	0.00	9.46	0.68	0.03	0.04	1.899	0.017	
	C	9+	7.35	0.09	3.89	—	0.04	0.04	1.899	0.019	
8	C	0-5	6.70	0.52	3.24	—	0.05	0.05	2.307	0.020	80.0
9	O	0-3	5.96	0.00	8.80	0.57	0.07	0.07	3.327	0.021	22.4
	AC	3-14	6.70	0.36	3.75	—	0.04	0.04	2.103	0.019	
10	AY	0-18	6.50	0.04	12.64	0.62	0.02	0.11	4.669	0.005	22.4
	C	18+	6.50	0.07	12.50	—	0.04	0.08	3.726	0.010	
11	AC	0-3	6.00	0.91	16.00	—	0.03	0.08	3.621	0.009	80.0
	C	3+	6.30	1.03	15.58	—	0.02	0.09	3.831	0.005	
12	AY	0-25	6.15	0.23	17.68	0.8	0.02	0.10	4.250	0.005	30.0
	C	25+	6.11	0.22	10.00	—	0.02	0.07	3.097	0.006	

N o t e. A — site; B —horizon; C —power, cm; D — pH_{water}; E — CO_2, %; F — C_{org}, %; G — S_{ha}/C_{fa}; H — basal respiration, µg CO_2-C/g·h^{-1}; I —SIR (substrate-induced respiration), µg CO_2-C/g·h^{-1}; J — C_{mic}, µg C/g soil; K — qCO_2, µg CO_2-c/mg C_{mic}·h^{-1}; L — rockiness, %; C_{org} — the content of total organic carbon, C_{ha} — carbon content of humic acids, C_{fa} — carbon content of fulvic acids, C_{mic} — carbon content of microbial biomass, qCO_2 — metabolic coefficient. Dashes mean that the analysis was not carried out.
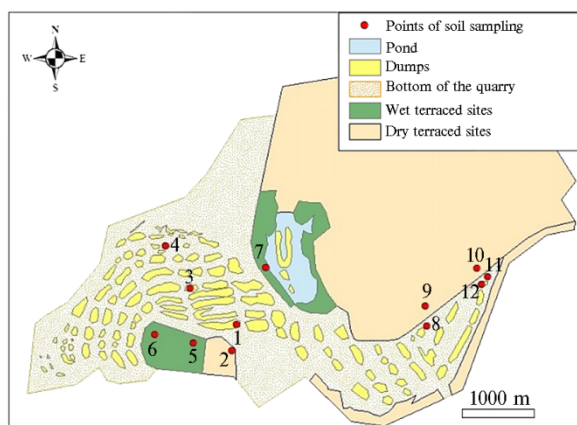

Fig. 1. The scheme of collection of soil samples in limestone quarry Pechurki: 1 — dump, rare vegetation (28 years old), 2 — dry terrace, small pine forest (16 years old), 3 — dump, rare vegetation (30 years old), 4 — dump, rare vegetation (8 years old), 5 — wet terrace, parvifoliate forest (35 years old), 6 — wet terrace, parvifoliate forest (35 years old), 7 — wet terrace, pleurocarpous moss pine forest (35 years old), 8, 11, 12 — base of the dump, rare vegetation (29 years old), 9, 10 — dry terrace, rare vegetation (29 years old) (Slantsevsky District, Leningrad Province, 2016).

accumulative ecotopes with optimal moisture conditions and physical parameters of the substrate. Differences between the upper organogenic horizons and the lower ones were increasing with the increase in the period of colonization. The primary substrate and the underlying horizons were characterized by higher acidity values (pH from 6 to 7.45) due to a large amount of primary minerals and the absence of organic matter. The content of organic carbon increased with the time of vegetation, while the pH decreased to 5.02. The most acidic reaction was observed on the positive elements of the relief (sections 1, 2, 4). The main dominants were *Populus tremula* and *Betula*

pendula.

The soils with a fulvate-humate type of humus prevailed, which was typical for the region. The increased proportion of humic acids is due to the carbonate substrate of the soil-forming rock. Five environmental factors that have the greatest influence on the distribution of vegetation have been highlighted by forward selection for inclusion in the model: the grain-size composition of the soil, rockiness, the content of physical clay, pH of water extract and moisture. These data are consistent with studies conducted in Canada [41]. All identified factors have a significant influence on the distribution of the plant community, but other sources note the great importance of the age of the dump [41-43].
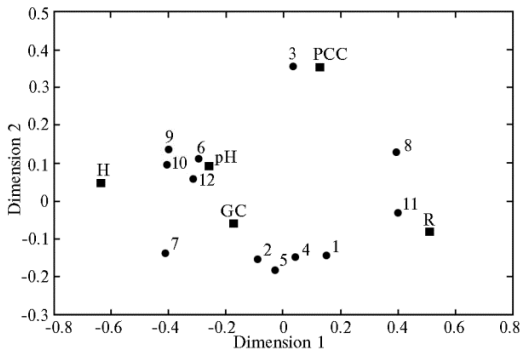

Fig. 2. Results of the canonical correspondence analysis of environmental factors and plant communities (non-metric multidimensional scales). Circles specify plant communities, PCC — physical clay content, H — humidity, GC — grain-size composition, R — rockiness (analysis with Statistica 7 software; quarry Pechurki, Slantsevsky District, Leningrad Province, 2016).

The results of the canonical correspondence analysis are shown in Figure 2. The first axis explains 65% variability, the second axis is for 23%. Sites with the maximum rockiness were colonized mainly by thinned plant groups with a predominance of *Ceratodon purpureus and Bryum pseudotriquetrum*. Many studies have confirmed that mosses and lichens are the first that colonize abandoned quarries [17, 44, 45]. Presumably, this is due to the fact that they are better adapted to adverse environmental conditions, and are also able to prepare a substrate for other plants [46-48]. Sites with a high proportion of clay fraction were colonized by grass spruce forest. Drained sandy loamy sites were occupied by various types of pine forests. Parvifoliate forests prevailed on the sites with a significant amount of skeletal material and a relatively low content of fine earth.

A total of 136 species of higher plants belonging to 106 genera, 49 families, 45 orders, 5 classes and 4 divisions were found in the quarry. In terms of the number of species, the *Fabaceae* and *Poaceae* families were predominant, each of them included 13 species (almost 10% of the diversity), as well as *Asteraceae* and *Rosaceae*, which included 11 species (8% each). A large number of *Fabaceae* family representatives are typical for disturbed habitats. *Asteraceae* and *Rosaceae* are the leading families of the flora of the Leningrad Region. There were 24 single-species families. A rather large number of species was in the *Orchidaceae* family, 9 species (6% of all species), which is due to the carbonate substrate of the quarry. A total of 22 types of mosses from 10 families and 11 epilithic lichens from 7 families, 75 species of algae and cyanobacteria from 38 genera and 29 families were also recorded. Representatives of all life forms of plants, common in the Leningrad REGION according to N.N. Tsvetlev, were found in the quarry [49]. There were all ecological groups in terms of requirements on soil richness: from oligotrophic plants to eutrophic and typical nitrophilous plants, as well as calciphile plants. All groups in terms of the water regime of soils have been identified, from xerophilous to hygrophytic plants. The number of higher vascular plants species on the sites varied from 14 to 39, depending on the ecotope. Fourteen protected species were found in different ecotopes. The maximum similarity of the species composition was observed at the rocky bottom of the quarry (the Sørensen-Czekanowski coefficient was 92%). The

most severe conditions for vegetation development were created here, so that communities were represented by a limited set of species. Sites with different positions in the relief were characterized by the smallest number of common species.

According to all the calculated indices, the smallest variety corresponded to the site where pine seedlings were planted on a flat surface (on a soft overburden) in 1970. Such biodiversity is typical for the pine forests of the Leningrad Province. The greatest variety, according to Shannon's index, corresponded to the recently recultivated site where the tree crowns had not yet closed, and marginal meadow species had not yet been displaced by typical forest species. By Simpson's index, the greatest variety was at the dumping ground of the quarry, where spontaneous succession without human intervention was taking place for more than 30 years. Biodiversity was reduced at flat sites under biological reclamation. Biodiversity has increased over time on sites, namely on dumps, where development was of spontaneous succession type. It is interesting that the development on heavily rocky sites (dumps of large fragments and rocky bottoms) was so slow that in 46 years no significant changes occurred. Apparently, this time is not enough for the natural transformation of the substrate by algae, lichens, and mosses. Our results are confirmed by the large body of literature data, which claims that spontaneous succession, in terms of the diversity of plant communities, is the best approach for partially managed land reclamation [50-53].

A microbiome is a link in the soil-plant system, which provides an ecological adaptation of the plant to stressful conditions of disturbed soils. In addition, microorganisms are able to utilize many chemical compounds by participating in the bioremediation of disturbed territories.

The content of microbial biomass in the samples studied ranged from 0.98 to 4.6 μg C/g of soil, while the values increased with the increase in the period of colonization and a tendency to a decrease in this indicator downward along the soil cross section was observed. According to published data [55, 56], the amount of microbial biomass can be considered as one of the indicators of organic matter accumulation and mineralization. Since basal respiration and the content of microbial biomass strongly depend on parameters such as humidity and temperature [57], a microbial metabolic coefficient, which refers to integral indicators of the biological state of soils, was calculated. Its values varied from 0.004 to 0.022 μg CO_2-C/mg $C_{mic} \cdot h^{-1}$. The obtained results indicate a reduced stability of microbial communities and the ineffective use of the organic substrate, especially at the first stages of quarry colonization. According to J. Frouz et al. [58], who actively studied the microbiological state of quarries in the Czech Republic, the index of soil respiration per unit of microbial biomass decreases with the increase in the period of colonization. However, according to the our data, it is difficult to identify the tendency of changes in microbiological indicators with age. Also, the authors note that the majority of microbiological indicators in 30-40-year-old communities were the same as in undisturbed communities.

The taxonomic analysis of microbiomes in our study showed that the dominant phylotype in all samples are *Proteobacteria* (55.7%), followed by *Actinobacteria* (17.0%), *Bacteroidetes* (10.3%), *Acidobacteria* (6.4%) and *Chloroflexi* (3.8%). The obtained results are consistent with the literature data: the listed phyla become dominant in the soils of disturbed habitats [25, 59, 60].

According to the results of the Principal Component Analysis (PCoA), the age of the dumps was the strongest factor (19.9% of the explained variability). The microbiomes of the old (35 years or more) dumps were distinctly isolated, while microorganism communities of young (8-16 years) and middle-aged (28-30 years) dumps tended to group into separate clusters (Fig. 3). Microbiomes
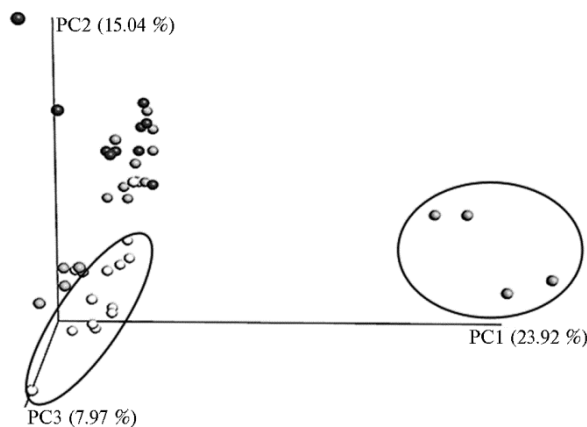
Fig. 3. The results of Principal Component Analysis (PCoA): white circles — old dumps (35 years old), gray circles — middle age dumps (28-30 years), black circles — young dumps (8-16 years). The highlighted areas specify groups of dumps of a certain age combined into clusters (the analysis was carried out with QIIME program; the Pechurki quarry, Slantsevsky District, Leningrad Province, 2016).

of the quarry bottom also formed a separate group, which was the result of special environmental conditions due to the rockiness of the substrate and thinned vegetation cover. The dominant taxa in young dumps were *Acinetobacter* (8.8% of the total community), *Micrococcaceae* (8%) and *Pseudomonas* (6%), while the number of these bacteria significantly decreased ($p < 0.05$) in the young > middle age > old dumps row. The *Acinetobacter* species have a comparatively wide ecological niche and can be found in habitats contaminated with hydrocarbons, activated sludge, sewage, and also in dumps with overburden grounds [61]. A high proportion of *Pseudomonadaceae* was recorded during the analysis of 6-year-old technosoils formed on dumps due to coal mining [62]. Representatives of *Micrococcaceae* (4.5%) and *Sphingomonadaceae* (1.4%) prevailed in the middle-aged dumps. The proportion of α-proteobacteria of the *Rhizobiales* order in the microbiome structure increased with the increase in the age of the dumps, mainly due to *Bradyrhizobiaceae* and *Hyphomicrobiaceae*. Statistically significant maxima of the proportion of these groups were in 35-year dumps — 5.0 and 2.5% of the sequences, respectively. It is known that the *Hyphomicrobiaceae* and *Bradyrhizobiaceae* bacteria play an important role in the processes of carbon and nitrogen transformations in soil [63]; many of them are often found in the rhizosphere of herbaceous plants and are part of the plant-growth-promoting (PGPR) group of bacteria. Old dumps were characterized by a significant (6-8-fold) increase in the proportion of *Micromonosporaceae* and *Sinobacteraceae* representatives in comparison with young and middle age dumps, and a significant increase in the proportion of *Chitinophagaceae* and *Cytophagaceae* bacteria in the microbiomes of 35 years dumps was also noted.

Wet terraces communities also differed somewhat from microbiomes of dry terraces and dump bases; however, no significant differences could be identified. Acidity (pH), which is one of the main soil factors and the main predictor of the microbial community composition [64-66], also did not have a significant effect on the composition of the microbiomes under study. Apparently, the formation of the specific structure of the microbial communities was the result of the combined effect of factors with their most favorable combination in 35-year-old dumps.

The results of a taxonomic analysis of the composition of dump microbiomes revealed a distinct change in the species composition of microorganisms with the increase in the age of dumps. Microorganisms that belong to the ecological group of copiotrophs, the presence of which in the soil marks the early stages of succession, prevailed in young dumps. According to the data of Y. Li et al. [25], the most important stage of microbiome recovery is between years 15 and years 20 after the beginning of the reclamation. In our studies, beginning with

middle-aged dumps (over 16 years old), oligotrophic groups of bacteria predominated in the community, including bacteria involved in the decomposition of complex biopolymers. The dominance of this group of microorganisms in the community can characterize the microbiocenosis transition to the climax stage.

Thus, the unfavorable grain-size composition becomes a limiting factor in the development of vegetation due to the high rockiness of rocky bottoms. The presence of areas with different physical parameters of the substrate, which are at different stages of vegetation, contributes to the development of a high diversity of plant communities. A change in the species composition of microorganisms is observed in the succession process, while the copiotrophic groups predominate in the early stages and the oligotrophic groups prevaile in the later stages. Monitoring of each site of the quarry is necessary in order to develop an optimal plan for territory reclamation and its further use for agricultural purposes since edaphic conditions can differ depending on the position in the relief and the impact on the ground. Territory reclamation for its further use for agricultural purposes requires expensive measures to flatten and level the high heterogeneity of the substrate. In terms of biodiversity, the best method for reclamation of carbonate quarries is the creation of favorable physical conditions of the substrate and further self-organized vegetation of the site.

R E F E R E N C E S

1. Williamson N.A., Johnson M.S., Bradshaw A.D. *Mine wastes reclamation.* Mining Journal Books, Ltd., London, United Kingdom, 1982.
2. Abakumov E., Trubetskoj O., Demin D., Celi L., Cerli C., Trubetskaya O. Humic acid characteristics in podzol soil chronosequence. *Chem. Ecol.*, 2010, 26: 59-66 (doi: 10.1080/02757540.2010.497758).
3. Abakumov E.V., Maksimova E.I., Lagoda A.V., Koptseva E.M. Soil formation in the quarries for limestone and clay production in the Ukhta region. *Eurasian Soil Science*, 2011, 44(4): 380-385 (doi: 10.1134/S1064229311040028).
4. Isachenko G.A., Reznikov I.A. *Biosfera*, 2014, 2: 196-200 (in Russ.).
5. Abakumov E.V., Cajthaml T., Brus J., Frouz J. Humus accumulation, humification, and humic acid composition in soils of two post-mining chronosequences after coal mining. *J. Soils Sediments*, 2013, 13(3): 491-500 (doi: 10.1007/s11368-012-0579-9).
6. Johnson N., Payton P., Spalding A. *The conservation value of metalliferous mine sites in Cornwall.* Cornwall Archaeological Unit and Institute of Cornish Studies, Truro, 1996.
7. Key R. Bare ground and the conservation of invertebrates. *British Wildlife*, 2000, 11: 183-191.
8. Androkhanov V.A., Ovsyannikova S.V., Kurachev V.M. *Tekhnozemy: svoistva, rezhimy, funktsionirovanie* [Technozems: properties, modes, functioning]. Novosibirsk, 2000 (in Russ.).
9. Archegova I.B. *Vestnik instituta biologii Komi nauchnogo tsentra Ural'skogo otdeleniya RAN*, 2013, 3(179): 24-27 (in Russ.).
10. Kuráž V., Frouz J., Kuráž M., Mako A., Shustr V., Cejpek J., Romanov O.V., Abakumov E.V. Changes in some physical properties of soils in the chronosequence of self-overgrown dumps of the Sokolov quarry-dump complex, Czechia. *Eurasian Soil Science*, 2012, 45(3): 266-272 (doi: 10.1134/S1064229312030076).
11. Andronov E.E., Petrova S.N., Pinaev A.G., Pershina E.V., Rakhimgalieva S.Zh., Akhmedenov K.M., Gorobets A.V., Sergaliev N.Kh. *Pochvovedenie*, 2012, 2: 173 (in Russ.).
12. Koptseva E.M. *Estestvennoe vosstanovlenie rastitel'nosti na tekhnogennykh mestoobitaniyakh Krainego Severa: Yamal'skii sektor Arktiki* [Natural restoration of vegetation on technogenic habitats of the Far North: Yamal sector of the Arctic]. St. Petersburg, 2005 (in Russ.).
13. Neshataev V.Yu., Karapukhin N.S., Efremov D.F., Knol' V.V., Neshataev M.V., Shtak K.D. *Prakticheskoe posobie po vosstanovleniyu rastitel'nogo pokrova na zemlyakh, narushennykh otkrytymi gornymi razrabotkami pri osvoenii mestorozhdenii poleznykh iskopaemykh v usloviyakh Kamchatskogo* kraya [Recommendation on restoration of vegetation on lands destroyed by open mining of minerals in Kamchatka territory]. St. Petersburg, 2012 (in Russ.).
14. Sumina O.I. *Formirovanie rastitel'nosti na tekhnogennykh mestoobitaniyakh Krainego Severa Rossii. Avtoreferat doktorskoi dissertatsii* [Formation of vegetation on technogenic habitats of the Russian Far North. DSci Thesis]. St. Petersburg, 2011 (in Russ.).
15. Abakumov E.V., Gagarina E.I. *Pochvoobrazovanie v posttekhnogennykh ekosistemakh kar'erov na Severo-Zapade Russkoi ravniny* [Soil genesis in post-technogenic of quarries in the North-West of the Russian Plain]. St. Petersburg, 2006 (in Russ.).

16. Yarwood S., Wick A., Williams M., Daniels W.L. Parent material and vegetation influence soil microbial community structure following 30-years of rock weathering and pedogenesis. *Microb. Ecol.*, 2015, 69(2): 383-394 (doi: 10.1007/s00248-014-0523-1).

17. Labbé L., Lanctôt B., Fortin, J.A. *Étalement des îlots d'aulnes et enrichis sement dessolsau complexe La Grande. Rapport pour la Vice-présidence Environnement, Hydro-Québec*. Institut de recherche en biologie végétale de Montréal, Montréal, Qué., 1995.

18. Laitinen J., Rehell S., Oksanen J. Community and species responses to water level fluctuations with reference to soil layers in different habitats of mid-boreal mire complexes. *Plant Ecol.*, 2008, 194(1): 17-36 (doi: 10.1007/s11258-007-9271-3).

19. Gretarsdottir J., Aradottir A.L., Vandvik V., Heegaard E., Birks H.J.B. Long-term effects of reclamation treatments on plant succession in Iceland. *Restor. Ecol.*, 2004, 12(2): 268-278 (doi: 10.1111/j.1061-2971.2004.00371.x).

20. Tormo J., Bochet E., Garcia-Fayos P. Roadfill revegetation in semiarid Mediterranean environments. Part II: Topsoiling, species selection, and hydroseeding. *Restor. Ecol.*, 2007, 15(1): 97-102 (doi: 10.1111/j.1526-100X.2006.00194.x).

21. Stifeev A.I., Golovastikova A.V., Bessonova E.A. *Vestnik Kurskoi gosudarstvennoi sel'skokhozyaistvennoi akademii*, 2011, 4(4): 40-41 (in Russ.).

22. Dangi S.R., Stahl P.D., Wick A.F., Ingram L.J., Buyer J.S. Soil microbial community recovery in reclaimed soils on a surface coal mine site. *Soil Sci. Soc. Am. J.*, 2012, 76(3): 915-924 (doi: 10.2136/sssaj2011.0288).

23. Acosta-Martinez V., Dowd S.E., Sun Y., Wester D., Allen V. Pyrosequencing analysis for characterization of soil bacterial populations as affected by an integrated livestock-cotton production system. *Appl. Soil Ecol.*, 2010, 45(1): 13-25 (doi: 10.1016/j.apsoil.2010.01.005).

24. Liu S., Liu W., Yang M., Zhou L., Liang H. The genetic diversity of soil bacteria affected by phytoremediation in a typical barren rare earth mined site of South China. *SpringerPlus*, 2016, 5(1): 1131 (doi: 10.1186/s40064-016-2814-0).

25. Li Y., Wen H., Chen L., Yin T. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. *PLoS ONE*, 2014, 9(12): e115024 (doi: 10.1371/journal.pone.0115024).

26. Luna L., Pastorelli R., Bastida F. The combination of quarry restoration strategies in semiarid climate induces different responses in biochemical and microbiological soil properties. *Appl. Soil Ecol.*, 2016, 107: 33-47 (doi: 10.1016/j.apsoil.2016.05.006).

27. Polyanskaya L.M. *Mikrobnaya suktsessiya v pochve. Avtoreferat doktorskoi dissertatsii* [Microbila cussession in soil. DSci Thesis]. Moscow, 1998 (in Russ.).

28. Fierer N., Leff J.W., Adams B.J., Nielsen U.N., Bates S.T., Lauber C.L., Owens S., Gilberte J.A., Wall D.H., Caporaso J.G. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *PNAS USA*, 2012, 109(52): 21390-21395 (doi: 10.1073/pnas.1215210110).

29. Zhang C., Xue S., Liu G.B., Zhang C.S. Effects of slope aspect on soil chemical and microbial properties during natural recovery on abandoned cropland in the Loess plateau, China. *Progress in Environmental Science and Engineering*, 2011, 356-360: 2422-2429 (doi: 10.4028/www.scientific.net/AMR.356-360.2422).

30. Shaposhnikov A.I., Belimov A.A., Kravchenko L.V., Vivanco J.M. Interaction of rhizosphere bacteria with plants: mechanisms of formation and factors of efficiency in associative symbiosis (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 3: 16-22 (in Russ.).

31. Burns R.G., DeForest J.L, Marxsen J., Sinsabaugh R.L., Stromberger M.E., Wallenstein M.D., Weintraub M.N., Zoppini A. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.*, 2013, 58: 216-234 (doi: 10.1016/j.soilbio.2012.11.009).

32. Ananyeva N.D., Susyan E.A., Chernova O.V., Wirth S. Microbial respiration activities of soils from different climatic regions of European Russia. *Eur. J. Soil Biol.*, 2008, 2: 147-157.

33. Tsitovich I.K. *Kurs analiticheskoi khimii* [Analytical chemistry]. Moscow, 1994 (in Russ.).

34. Rastvorova O.G. *Fizika pochv (prakticheskoe rukovodstvo)* [Soil physics]. Leningrad, 1983 (in Russ.).

35. Ponomareva V.V., Plotnikova T.A. V knige: *Problemy pochvovedeniya* /Pod redaktsiei M.A. Glazovskoi, I.A. Sokolova [Pedology. M.A. Glazovskaya, I.A. Sokolov (eds.)]. Moscow, 1978: 65-72 (in Russ.).

36. Anderson J.P.E., Domsch K.H.A. Physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.*, 1978, 10(3): 215-221 (doi: 10.1016/0038-0717(78)90099-8).

37. Blanchet F.G., Legendre P., Borcard D. Forward selection of explanatory variables. *Ecology*, 2008, 89(9): 2623-2632 (doi: 10.1890/07-0986.1).

38. Ter Braak C.J.F. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 1986, 67(5): 1167-1179 (doi: 10.2307/1938672).

39. Edgar R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 2010, 26(19): 2460-2461 (doi: 10.1093/bioinformatics/btq461).

40. Huber J.A., Butterfield D.A., Baross J.A. Temporal changes in archaeal diversity and chemistry

in a mid-ocean ridge subsea floor habitat. *Appl. Environ. Microb.*, 2002, 68: 1585 (doi: 10.1128/AEM.68.4.1585-1594.2002).

41. Hugron S., Andersen R., Poulin M., Rochefort L. Natural plant colonization of borrow pits in boreal forest highlands of eastern Canada. *Botany*, 2011, 89: 451-465 (doi: 10.1139/B11-036).

42. Gilardelli F., Sgorbati S., Armiraglio S., Citterio S., Gentili R.. Assigning plant communities to a successional phase: time trends in abandoned limestone quarries. *Plant Biosyst.*, 2016, 150(4): 799-808 (doi: 10.1080/11263504.2015.1011722).

43. Řehounková K., Prach K. Spontaneous vegetation succession in gravel—sand pits: a potential for restoration. *Restor. Ecol.*, 2008, 2(16): 305-312 (doi: 10.1111/j.1526-100X.2007.00316.x).

44. Karim M.N., Mallik A.U. Roadside revegetation by native plants: I. Roadside microhabitats, floristic zonation and species traits. *Ecol. Eng.*, 2008, 32(3): 222-237 (doi: 10.1016/j.ecoleng.2007.11.003).

45. Kershaw G.P., Kershaw L.J. Successful plant colonizers on disturbances in tundra areas of northwestern Canada. *Arctic Alpine Res.*, 1987, 19(4): 451-460 (doi: 10.2307/1551411).

46. Alpert P. The discovery, scope, and puzzle of desiccation tolerance in plants. *Plant Ecol.*, 2000, 151(1): 5-17 (doi: 10.1023/A:1026513800380).

47. Beckett R.P., Kranner I., Minibayeva F.V. Stress physiology and the symbiosis. In: *Lichen biology. 2nd ed.* T.H. Nash, III (ed.). Cambridge University Press, Cambridge, UK, 2008.

48. Sancho L.G., de la Torre R., Horneck G., Ascaso C., de los Rios A., Pintado A., Wierzchos J., Schuster M. Lichens survive in space: results from the 2005 LICHENS experiment. *Astrobiology*, 2007, 7(3): 443-454 (doi: 10.1089/ast.2006.0046).

49. Tsvelev N.N. *Opredelitel' sosudistykh rastenii Severo-Zapadnoi Rossii (Leningradskaya, Pskovskaya i Novgorodskaya oblasti)* [Identification keys of vascular plants in the North-West of the Russia (Leningradm Pskov, and Novgorod regions]. St. Petersburg, 2000 (in Russ.).

50. Hodačová D, Prach K. Spoil heaps from brown coal mining: technical reclamation vs spontaneous revegetation. *Restor. Ecol.*, 2003, 11: 385-391 (doi: 10.1046/j.1526-100X.2003.00202.x).

51. Mudrák O., Frouz J., Velichová V. Underestory vegetation in reclaimed and unreclaimed post-mining forest stands. *Ecol. Eng.*, 2010, 36: 783-790 (doi: 10.1016/j.ecoleng.2010.02.003).

52. Šebelíková L., Řehounková K., Prach K. Spontaneous regeneration vs. forestry reclamation in post-mining sand pits. *Environ. Sci. Pollut. Res.*, 2016, 23: 13598-13605.

53. Tropek R., Černá I., Straka J., Čížek O., Konvička M. Is coal combustion the last chance for vanishing insects of inland drift sand dunes in Europe? *Biol. Conserv.*, 2013, 162: 60-64 (doi: 10.1016/j.biocon.2013.03.027).

54. Archegova I.B. *Pochvovedenie*, 1992, 1: 58-64 (in Russ.).

55. Anan'eva N.D. *Mikrobiologicheskie aspekty samoochishcheniya i ustoichivosti pochv* [Microbiological aspects of soil self-restoration and sustainability]. Moscow, 2003 (in Russ.).

56. Jenkinson D.S., Ladd J.N. Microbial biomass in soil: measurement and turnover. *Soil Biochemistry*, 1981, 5: 415-471.

57. Prikhod'ko V.E., Sizemskaya M.L. *Pochvovedenie*, 2015, 8: 974 (in Russ.).

58. Frouz J., Nováková A. Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development. *Geoderma*, 2005, 129: 54-64 (doi: 10.1016/j.geoderma.2004.12.033).

59. Mummey D.L., Stahl P.D., Buyer J.S. Soil microbiological properties 20 years after surface mine reclamation: spatial analysis or reclaimed and undisturbed sites. *Soil Biol. Biochem.*, 2002, 34: 1717-1725 (doi: 10.1016/S0038-0717(02)00158-X).

60. Megharaj M., Naidu R., Ramadass K., Samkumar R.A., Satheesh V., Subashchandrabose S.R., Thavamani P., Venkateswarlu K. Microbes from mined sites: Harnessing their potential for reclamation of derelict mine sites. *Environ. Pollut.*, 2017, 230: 495-505 (doi: 10.1016/j.envpol.2017.06.056).

61. Doughari H.J., Ndakidemi P.A., Human I.S., Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes Environ.*, 2011, 26: 101-112 (doi: 10.1264/jsme2.ME10179).

62. Urbanova M., Kopecky J., Valaskova V., Mareckova M.S., Elhottova D., Kyselkova M., Loccoz Y.M., Baldrian P. Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining. *FEMS Microbiol. Ecol.*, 2011, 78: 59-69 (doi: 10.1111/j.1574-6941.2011.01164.x).

63. Andronov E.E., Ivanova E.A., Pershina E.V., Orlova O.V., Kruglov Yu.V., Belimov A.A., Tikhonovich I.A. *Byulleten' Pochvennogo instituta im. V.V. Dokuchaeva*, 2015, 80: 83-94 (in Russ.).

64. 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. *Appl. Environ. Microb.*, 2009, 75: 5111-5120 (doi: 10.1128/AEM.00335-09).

65. Kaiser K., Wemheuer B., Korolkow V., Wemheuer F., Nacke H., Schöning I. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports*, 2016, 6: 33696 (doi: 10.1038/srep33696).

66. Rousk J., Beeth E., Brookes P.C., Lauber C.L., Lozupone C., Caporaso J.G. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal*, 2010, 4: 1340-1351 (doi: 10.1038/ismej.2010.58).