1. Contestant profile

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▪ University / Organisation	Saint-Petersburg State University[1], All-Russian Research Institute of Agricultural Microbiology[2], A.P. Karpinsky Russian Geological Research Institute[3],
▪ Number of people in your team:	**3**

2. Project overview

Title:	Biological activity and diversity of microbial communities of the Pechurki quarry
Contest: (Research/Community)	Research
Quarry name:	Pechurki

Abstract (max 0.5 page)

Former limestone quarry was investigated with aim to to study the soil microbiome in the process of succession on dumps of various ages under natural self-growth and recultivation of the limestone quarry "Pechurki", as well as the popularization of microbiological studies. 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. 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. 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. We proposed a new idea of land reclamation. We did a lot of work aimed at involving local residents in our project and popularizing microbiology. We branded our popular science blog and prepared a popular science brochure, designed the design and made drawings.

Introduction

Soil microorganisms are the main agents that conduct a cycle of substances and support the homeostasis of ecosystems on Earth. According to the principles of soil microzonality, the microbial pool and the concept of duplication of ecological functions by different microorganisms, homeostasis is carried out very accurately provided the microbial diversity is preserved. All biogeochemical processes in the biosphere over the course of several billion years were determined only by prokaryotes. Bacteria have unique functions that only prokaryotes can do: nitrogen fixation, methanogenesis and sulfate reduction. In connection with the increasing anthropogenic pressure on the biosphere, leading to a decrease in biodiversity, the issue of preserving bacterial diversity of soils, as the main factor for maintaining homeostasis and ecosystem sustainability, acquires special significance.

Despite the fact that the microbial communities play a huge role in maintaining the stability of ecosystems, the circulation of substances, the restoration of the organic profile of soils, the optimization of its physical and chemical characteristics, and the fact that the microbiological activity of soils is recognized as a universal indicator of the state of soils, the state of microbiocenosis of man-made ecosystems is very poorly studied. The increase in the microbiological activity of quarries and the study of its qualitative composition with the aim of accelerating the processes of primary succession is a particularly urgent task.

According to the principle of systemicity, the successful functioning of ecosystems requires the presence of three main components, namely the plant community, the substrate or soil and the binding component - the microbiome. Previously, the "Biodiversity in space and time" team studied in detail the soil and vegetation cover of the quarry, determined the agrochemical and agrophysical parameters of the substrate for 12 different ecotopes of the quarry. They also argue that the level of microbiological activity of soils at the site is extremely low, this may hamper the restoration of ecosystems.

The purpose of this work was to study the soil microbiome in the process of succession on dumps of various ages under natural self-growth and recultivation of the limestone quarry "Pechurki", as well as the popularization of microbiological studies. To achieve the goal, the following tasks were set:

- ✓ to evaluate the alpha and beta diversity of microbial communities of various ecotopes of the quarry;
- ✓ to make chronoseries, assessing how diversity varies in time and space;
- ✓ to assess what environmental factors have a leading impact on the qualitative composition of the microbial community;
- ✓ to assess the microbiological activity of soils;
- ✓ to study the possibility of compiling lists of bacterial taxa, according to which it is possible to mark different stages of soil restoration;
- ✓ based on data on the microbiota of different sites, to propose activities aimed at increasing the sustainability of the ecosystem of the quarry;
- ✓ to assess microbiological contamination of soils.

Moreover in order to popularize microbiology, the following tasks were set:

- ✓ to organize lectures and seminars for schoolchildren, students and local residents;
- ✓ to organize a contest for "the most interesting issue";
- ✓ to carry out a joint experiment with children in the field;
- ✓ to carry out an excursion to the quarry;
- ✓ to participate in conferences;
- ✓ to publish of an article in the journal Agricultural Biology (abstracted and/or indexed in Scopus, WoS Clarivate Analytics);
- ✓ to develop of a science-popular brochures.

Methods

The investigation has been conducted on the Pechurki limestone quarry. The area is characterized by an Atlantic-continental climate (average annual temperature of 2°C and average annual precipitation of 700 mm per year, evaporation rate is about 450 mm). The climate characteristics are caused by the proximity to the sea, with moderately cold winters and warm summers. The natural soil cover of the region is mainly characterized by the predominance of spodisolsthat have developed on acid moraine tills or fluvioglacial deposits, but in region of Izhora Plateau also Calcaric Leptosols are typical due to the exposition of limestone derived parent materials of the surface (Ivlev 1994).

The main type of vegetation belongs to southern taiga with coniferous forests, but a high frequency of natural and anthropogenic disturbances has led to the establishment of small-leaved forests. Ecosystems of Izhora

Plateau also characterizes by presence some representative of sub boral flora due to increased content of carbonates in local parent materials.

The Pechurki quarry is situated in Slantsy Town in the Leningrad region. Production of limestone was stopped in 2014 but biological reclamation was initiated already in 1970 and, in the course of this work, pine was planted on the dumps. Therefore, there are now reclaimed plots at various stages of overgrowing. However, the vast majority of the quarry area has undergone spontaneous revegetation. First, we identified all of the landforms of the quarry. Soil samples were collected from 9 key plots (Appendix 1) formed at 9 different sites under common plant communities (Fig. 1), where A - UmbricLeptosols (Calcaric) under accumulative self-overgrowing ecotope, overgrowing age is about 35 years, B - Leptosols (Skeletic, Calcaric) under self-overgrowing bottom of the quarry, overgrowing age is 29 years. Since the small-scale topographical differences strongly affect the soil and vegetation succession dynamics (Burga et al. 2010), plots with different topographic features were chosen. Field descriptions of the soil pits were made.

Fig. 1. The most different surface of the quarry: A- Umbric Leptosols (Calcaric); B - Leptosols (Skeletic, Calcaric)

The following parameters were determined in soil samples: substrate-induced respiration (Ananyeva et al. 2008), basal soil respiration, organic carbon oxidation by the bichromate (Walkey and Blake) method, pH in water and salt suspension (1:2.5), acidometric evaluation of CO_2 content in carbonates (Tsitovich 1994), skeletal fraction content, particle size distribution of the soil using pipette-by Kaczynski with pyrophosphate peptization of microaggregates (Rastvorova 1983), fractional-group composition of humus by Tyurin's scheme modified by Ponomareva and Plotnikova with extraction of groups of humic and fulvic acids (Ponomareva and Plotnikova 1980), quantitative determination of carbon of microbial biomass (Cmic) according to the formula proposed by Anderson and Domsch (1978), Cmic (g C / g soil) = LED (L CO_2 / g soil per hour) × 40.04 + 0.37, microbial metabolic rate (the specific respiration microbial biomass, qCO2) is found as the ratio of basal respiration to microbial biomass carbon index: qCO2 (mg CO_2 / Cmic mg / h) = DB / Cmic.

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

The purified DNA preparations (10–15 ng) were used as templates in the PCR reaction (temperature profile: 95°C for 30 s, 50°C for 30 s, 72°C for 30 s, 30 cycles in total) using Encyclo polymerase (Eurogen, Russia) and universal primers to the V4 variable region of the 16S rRNA gene: F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGACTACVSGGGTATCTAAT) (Bates et al. 2010). The primers included oligonucleotide barcodes for each sample and the service sequences required for 454-pyrosequencing by «Roche» protocol (Roche, Switzerland). Sample preparation and sequencing were performed on a GS Junior device (Roche, Switzerland) according to the manufacturer's recommendations.

Downstream processing of the 16S rRNA gene libraries was performed using the QIIME software package (Caporaso et al. 2010). At the first stage, the sequence quality was verified: sequences smaller than 200 nucleotides in length, with a quality score of less than 25, containing incorrectly read primer and multiplex identifier sequences, as well as extended homopolymeric repeats (more than eight nucleotides) and unidentified nucleotides were excluded from the analysis. All non-bacterial and chimeric sequences were excluded from the analysis; the data were normalized according to the number of sequences in the library of the smallest size. As a result of all of these procedures, 14,312 sequences were selected. After the data normalization procedure, the number of sequences in each library was 4700. Sequences with similarity over 97% were combined into operational taxonomic units (OTU) using a *de novo* algorithm (based on the UCLUST method). From each OTU, one sequence was chosen to compose a set of representatives. The next stage was the classification of

representative sequences using the RDP naïve Bayesian rRNA Classifier program and PyNast algorithm alignment (Caporaso et al. 2010), and a specially designed set of sequences, the Greengenes core set (DeSantis et al. 2006), was used to align the sequences. The aligned sequences were used to construct the distance matrix and phylogenetic tree.

To assess biodiversity and conduct a comparative analysis of communities, the parameters of α- and β-diversity were calculated. The α-diversity was assessed using the index of species richness (the number of OTUs in the sample) and the Shannon index. The reliability of differences in α-diversity indices among microbiomes was assessed using the t-test. To assess the β-diversity, we used the weighted UniFrac method, which allows estimating the percentage of similarities/differences between all pairs of compared microbiomes (Lozupone and Knight 2005). To represent the results of the analysis, we used a multidimensional statistics approach (analysis of the main components) using the Emperor program.

To assess the reliability of differences in the representation of individual taxa in the analyzed samples, in addition to the QIIME software, a script was written using the Python programming language. This script performs multiple pairwise tests of contingency tables of OTU frequencies in different collections of samples/replicates. The algorithm dynamically chooses either the G-test or the Fisher exact test depending on the circumstances and applies Bonferroni p-value correction.

Results

The status of the microbial communities at the quarries is poorly understood. This is problematic because microbial communities play critical roles in maintaining the sustainability of communities and ecosystem development. Therefore, studies addressing the state of a microbiological component of man-made habitats is particularly relevant. The microbial biomass content ranged from 0.98 to 4.60 g C g^{-1} soil. Although we were unable to identify any trends in microbiological criteria based on the type of vegetation or landforms, values tended to increase with time of overgrowing. We detected a tendency for reduced microbiological activity from the upper layer to the parent material. Since the basal respiration and microbial biomass content depends largely on parameters such as humidity and temperature (Prihodko and Sizemskaya 2015), we calculated the microbial metabolic rate, which refers to integral biological indicators of soil. The values of this index ranged from 0.004 to 0.022 g CO_2 C g^{-1} h^{-1}. These results indicate a reduced stability of microbial communities and the inefficient use of the organic substrate, especially during the early stages of overgrowing.

According to the results, 55.7% of the total biodiversity belongs to the most numerous *Proteobacteria* group in the all examined sites, followed by the *Actinobacteria* (17.0%), followed by *Bacteroidetes* (10.3%), *Acidobacteria* (6.4% %), and *Chloroflexi* (3.8%).

By principal component analysis (PCA), the age of plots was identified as the most significant environmental factor (explaining 19.9% of the total variance) (Fig. 2). The microbial communities of the old (≥ 35-years-old) sites are clearly isolated, communities of microorganisms of young (8–16-years-old) and middle-aged (28–30-years-old) sites tend to group into separate clusters.

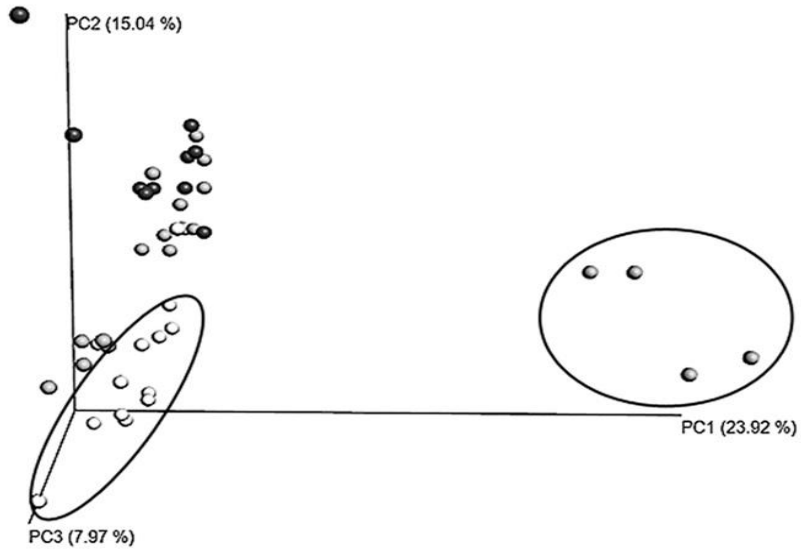


Figure 2. Principal component analysis. White circles – old sites (35 years), gray circles – middle-aged sites (28-30 years), black circles – young sites (8-16 years).

Compared to young and middle-aged sites, old sites are characterized by a significant (6–8 times) increase in representatives of *Micromonosporaceae* and *Sinobacteraceae*. Communities of young sites have more representatives of *Pseudomonas* (4.2-times) and *Micrococcaceae* (3.8-times) than the communities of old sites. The communities of wet terraces also differ from the microbiomes of the remaining ecotopes (dry terraces and the bases of the dumps). However, no significant differences could be identified in pH values, which is an important soil characteristic, had no significant effect on the composition of the microbial community. Taxonomic analysis of communities at the phyla level did not reveal differences between differently aged dumps. In all cases, the largest phyla were the *Proteobacteria* (55.7%), *Actinobacteria* (17.0%), *Bacteroidetes* (10.3%), *Acidobacteria* (6.4%), and *Chloroflexi* (3.8%). The dominant taxa in young dumps were *Acinetobacter* (8.8% of the total community), *Micrococcaceae* (8%) and *Pseudomonas* (6%). In the middle-aged sites, representatives of *Micrococcaceae* (4.5%) and *Sphingomonadaceae* (1.4%) prevailed. Old sites showed a high proportion of representatives of *Bradyrhizobiaceae* (5%),*Chitinophagaceae* (2.9%) and *Hyphomicrobiaceae*(2.5%).

The alpha diversity of sites of different ages was assessed (Fig.3). There is a tendency to increase species diversity and species richness with a period of overgrowing. However, these results are not statistically reliable.

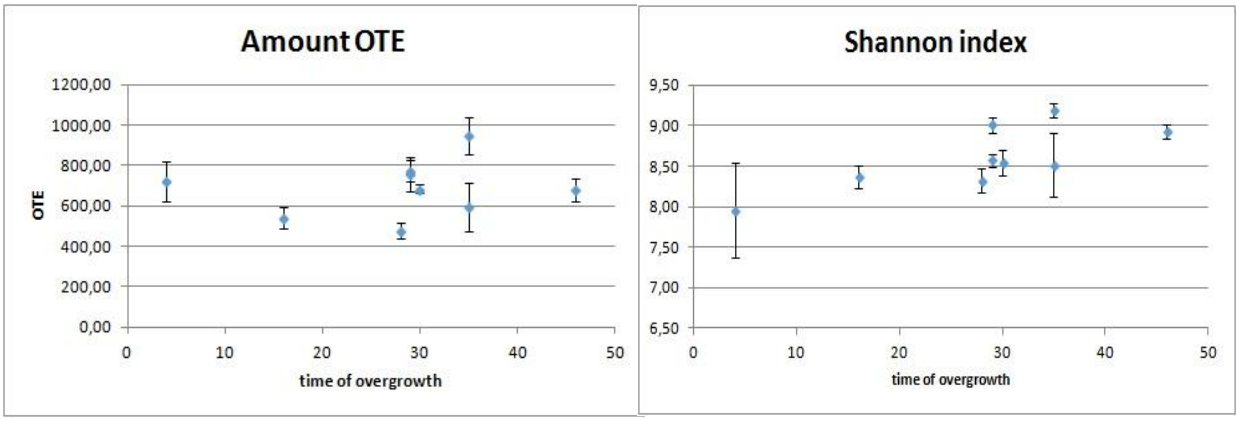


Figure 3. Number of species (OTE units) and Shannon index for different sites of the quarry

The number of OTE units found (an analog of the species) at the each site varies from 9364 to 9408. Currently there is no generally accepted methodology for assessing the change in the number of individual taxa. We proposed to evaluate the significant change in the proportion of taxa in the group (usually in pairs) of the samples. These taxa are called the "active group." The taxonomic structure of microbioms at the level of bacterial families (families representing more than 0.5% in the community and whose share is statistically significantly different between dumps of different ages) is presented in the Figure 4. According to the results of taxonomic analysis, the most *Acinetobacter* (8.8% of the total community), *Micrococcaceae* (8%) and *Pseudomonas* (6%) dominate at the young sites. Dominant taxa of middle-aged sites are *Micrococcaceae* (4.5%) and Sphingomonadaceae (1.4%). Interestingly, with the increase in the period of overgrowing, the participation of the alpha proteobacteria (*Rhizobiales*) is statistically significantly increased in the microbiom, mainly due to the families of *Bradyrhizobiaceae* (5% of the sequences) and *Hyphomicrobiaceae* (2.5% of the sequences). A distinctive feature of the bacteria of the families *Hyphomicrobiaceae* and *Bradyrhizobiaceae* is their significant role in the processes of transformation of carbon and nitrogen in the soil (Andronov et al., 2015). Many of them belong to a group of bacteria that have a set of plant-useful properties (Plant-Growth-Promoting (PGPR)) and are often found in the rhizosphere of herbaceous plants. In microbioms of 35-year-old sites, the proportion of such groups as *Micromonosporaceae* and *Sinobacteraceae* is increased at 6-8 times, and also significantly more bacteria of *Chitinophagaceae* and *Cytophagaceae*, compared with young and middle-aged sites. Microbial communities of sites with a shorter period of overgrowth are distinguished by a 4.2 times increase in the proportion of representatives of *Pseudomonas* and 3.8 times the number of *Micrococcaceae*, compared to relatively old-age sites. Interestingly, according to the decrease in the content of these bacteria in sites of different ages, it is possible to build a statistically significant ($p < 0.05$) series: young-medium-old sites. Communities of wet terraces differ slightly from other microbioms, but it was not possible to confirm this statistically. Interestingly, one of the main soil factors, as the pH reaction also did not have a significant effect on the composition of the microbial community. At the Phil level, there was no difference in the composition of the microbiom between the dumps with different maturation times.

at the 0.5% share

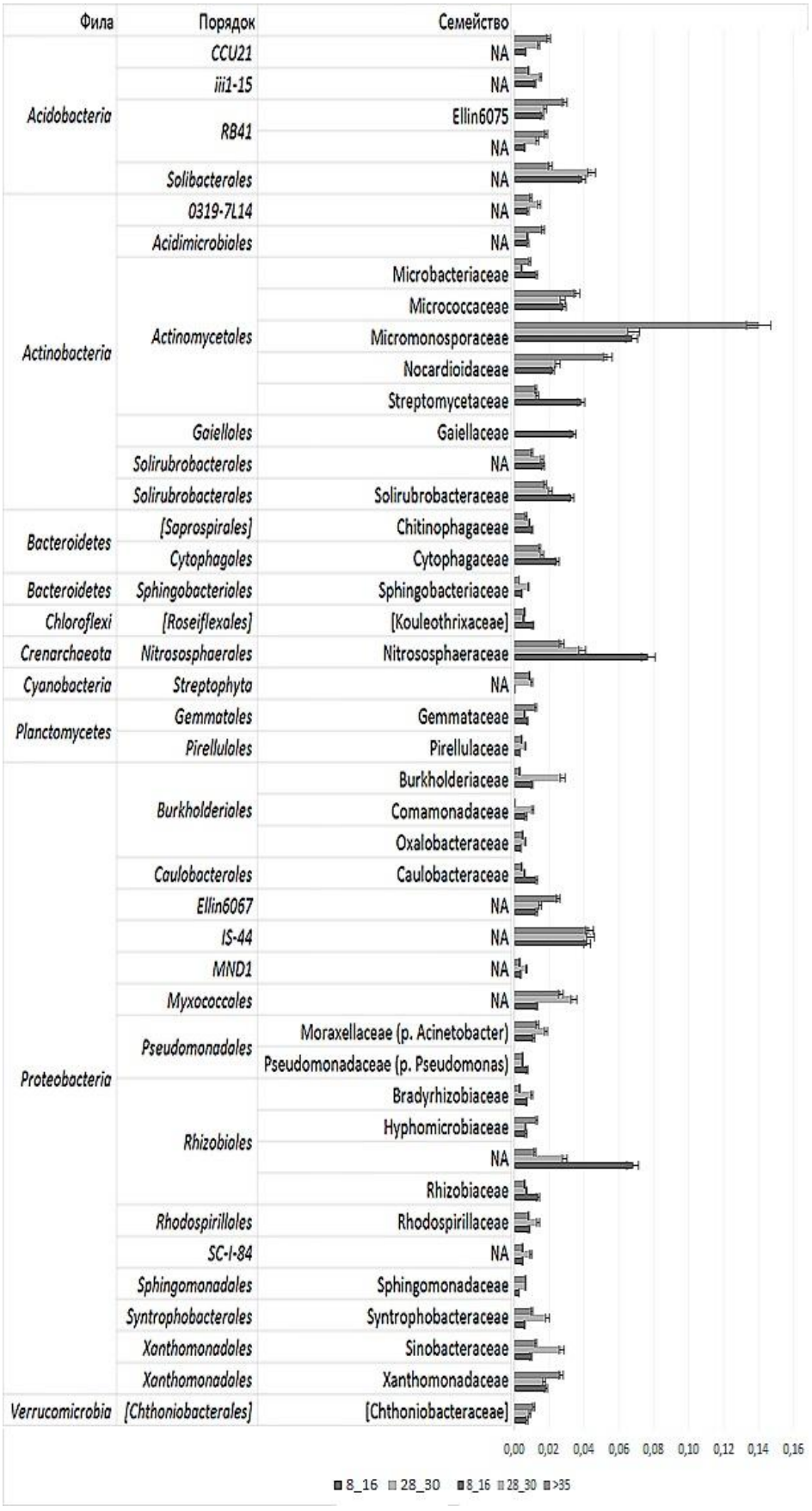


Figure 4. Taxonomic structure of microbioms level of bacterial families (families representing more than in the community are represented and the of which is statistically significantly different between dumps of different ages).

As a result, a distinct change in the species composition of microorganisms with the timing of overgrowth was revealed. As expected, the early stages of succession are dominated by representatives of the ecological group of copiotrophs (r-strategists). With the increase in the periods of overgrowing (starting from 16 years) oligotrophic groups of bacteria (K-stratedgists) appear in the dominants, the presence of which indicates the completeness of the carbon cycle and the stabilization of the community composition, as they participate in the decomposition of complex biopolymers and mark the transition of the community to the climax stage.

Discussion

We confirmed that the microbiological activity of the soils of the quarry is 46.3 times less than that of unaltered communities (Stolnikova et al., 2011). According to J. Frouz et al. (2005), who studied the microbiological state of quarries in the Czech Republic, the index of soil respiration per unit of microbial biomass decreases with increasing period of overgrowing. However, according to our data, it is difficult to identify the tendency of changes in microbiological activity with age. Also, the authors note that under the 30-40-year-old communities the majority of microbiological indicators were the same as in undisturbed communities, which also contradicts our results.

The number of microorganisms per gram of soil was 28 850 – 83 660 against about 970 000 for unbroken soils in the region (Mishustin, 1947). The received results testify to the lowered stability of microbial communities and ineffective use of organic substrate, especially at the first stages of overgrowth of the quarry.

Forty-four active groups were identified that significantly changed their numbers with the time of overgrowth, according to which, as we believe, it is possible to mark the stages of soil-restoration (Figure 4).

The urgent task is to increase the proportion of oligotrophs at the first stages of succession, since this will significantly increase the homeostasis of ecosystems in the rate of recovery of the soil profile. In all natural soils, due to the principle of conveyor processing of plant litter, R-strategists dominate the tier of the litter and the uppermost soil layer (Fig. 5). K-strategies (oligotrophic groups) responsible for the completeness of the carbon cycle, stabilization of the community composition, as they participate in the decomposition of complex biopolymers and marking the transition of the community to the climax stage, are located in the lower layers.

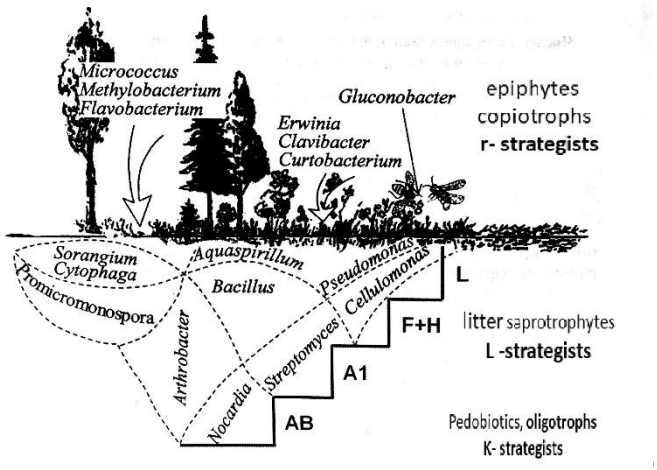


Figure 5. Distribution of bacteria along the tiers of forest biogeocenosis (Dobrovolskaya, 2002)

The existing reclamation technique involves an application of the topmost litter layer from adjacent undisturbed communities to the surface to speed up the restoration of forest ecosystems, or grass mowing in meadows and their subsequent distribution to a quarry for restoration of meadow communities (Appendix 2).

However, in this case to the fresh substrate get only r-strategists, which already dominate on the fresh areas (Figure 6). Based on the results of our research, we believe that it may be necessary to revise the recommendations on reclamation and selectively disperse on the surface of the reclamated areas the borax of the entire soil profile of undisturbed communities, and not only the upper layer, since in the underlying layers there are principally valuable species of bacteria. It is a cheap and safe process, that will significantly diversify microbiomas, increase the variety of different functional groups of prokaryotes, close the cycle. In conditions of man-caused habitats, the soil horizon does not yet have vertical stratum and all processes are concentrated in the uppermost layer of the substrate (up to 10 cm deep). The new approach assumes selection of several soil profile samples with a value of 1 meter from the donor undisturbed areas by means of a geological borax. In the case when it is planned to restore a meadow ecosystem, natural meadows should be selected as donor sites, while restoring forest ecosystems - forests. Then the selected samples must be mixed together with the mown grass from the donor meadow or forest litter, and applied to the reclaimed surface.

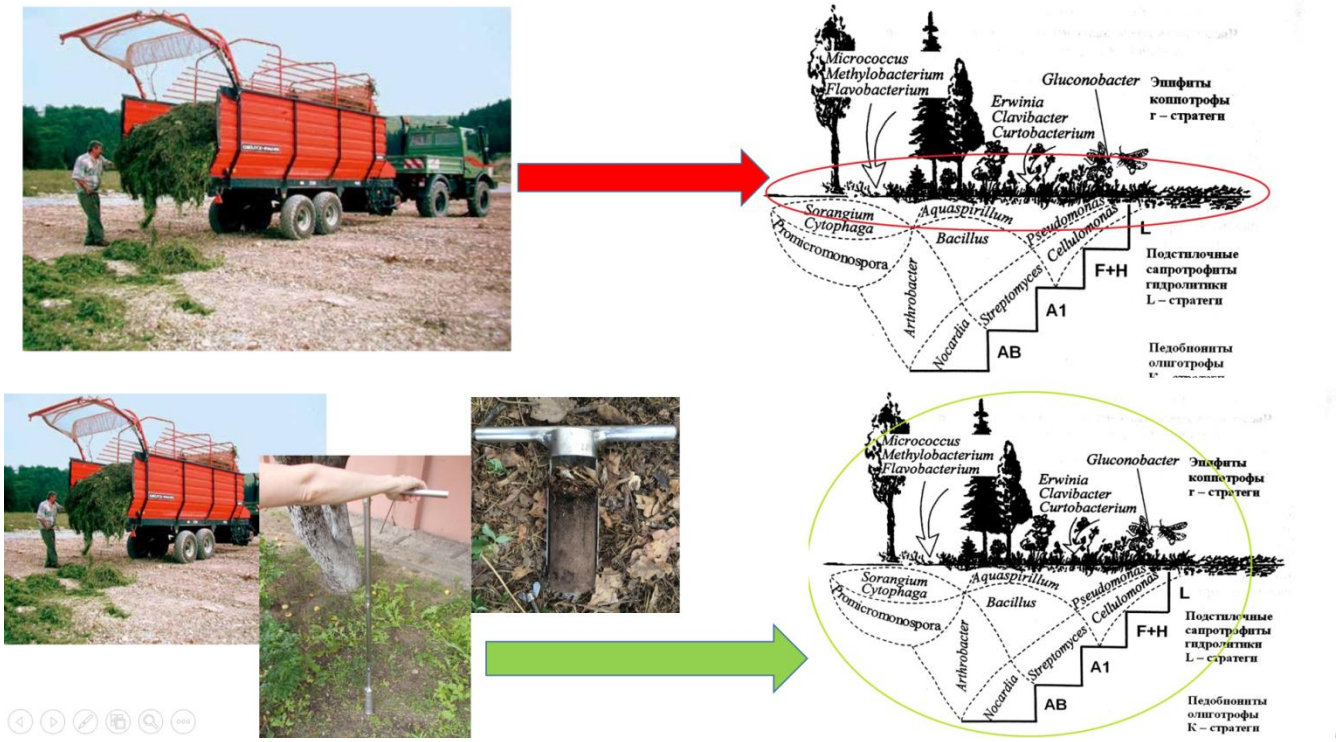


Figure 6. A scheme that reflects the difference in the microbiom in the case of a conventional method of reclamation and an improved

We plan to conduct a field experiment, where we will also add areas to control where only selected samples of soil borax and control plots without reclamation will be applied. If the experiment is successful, an improved method is planned to be patented.

The public work

To popularize microbiology, we conducted work on branding our project. The design and concept of the blog was developed, which we updated daily, not only in accessible scientific and popular language, but also in the more complicated questions that concerned the methods of our work. In addition to acquaintance with bacteria, we created our own original images (Fig. 7), which are collected in a single popular science book-brochure for a wide range of readers, which will continue to popularize scientific information about microbiology (application). Now the brochure is translated into English.

Figure 7. Illustration from since-popular brochure

In addition to the lecture for local residents, a contest was announced for the most interesting question in our blog, which they can ask us via e-mail. A joint field experiment was conducted with the children from the local library to determine the intensity of the decomposition of cellulose, its results reflected a very low level of soil biological activity, but the results were not included in the analysis. We conducted a paleontological excursion at the quarry with an emphasis on the role of bacteria in biogeochemical cycles (Fig 8).

Рисунок 8. Совместный полевой эксперимент и экскурсия на карьер

Together with the project "Biodiversity in space and time" we have published an article "Dynamics of the plant community and microbiome of chrono-series of the post-technology soil in limestone quarry in the conditions of recultivation" (doi: 10.15389 / agrobiology) in the journal Agricultural biology, that is indexed in leading scientific bases (Scopus, Web of Science).

We also prepared a report on the "Ecosystem Services of the soil microorganisms and microbiological components of Peachurki" (DOI: 10.17011 / conference / eccb2018 / 107863) at the European Conference on Computational Biology (ECCB 2018) .We made a presentation at Spring Campus 2018 (University Alliance for Sustainability) "Moving Beyond the Ivory Tower: How to Do Science and Universities", which was held this spring in Berlin. We delivered a report at a seminar for students and interested persons "Study and Do" , which was spent in the St Petersburg State University.

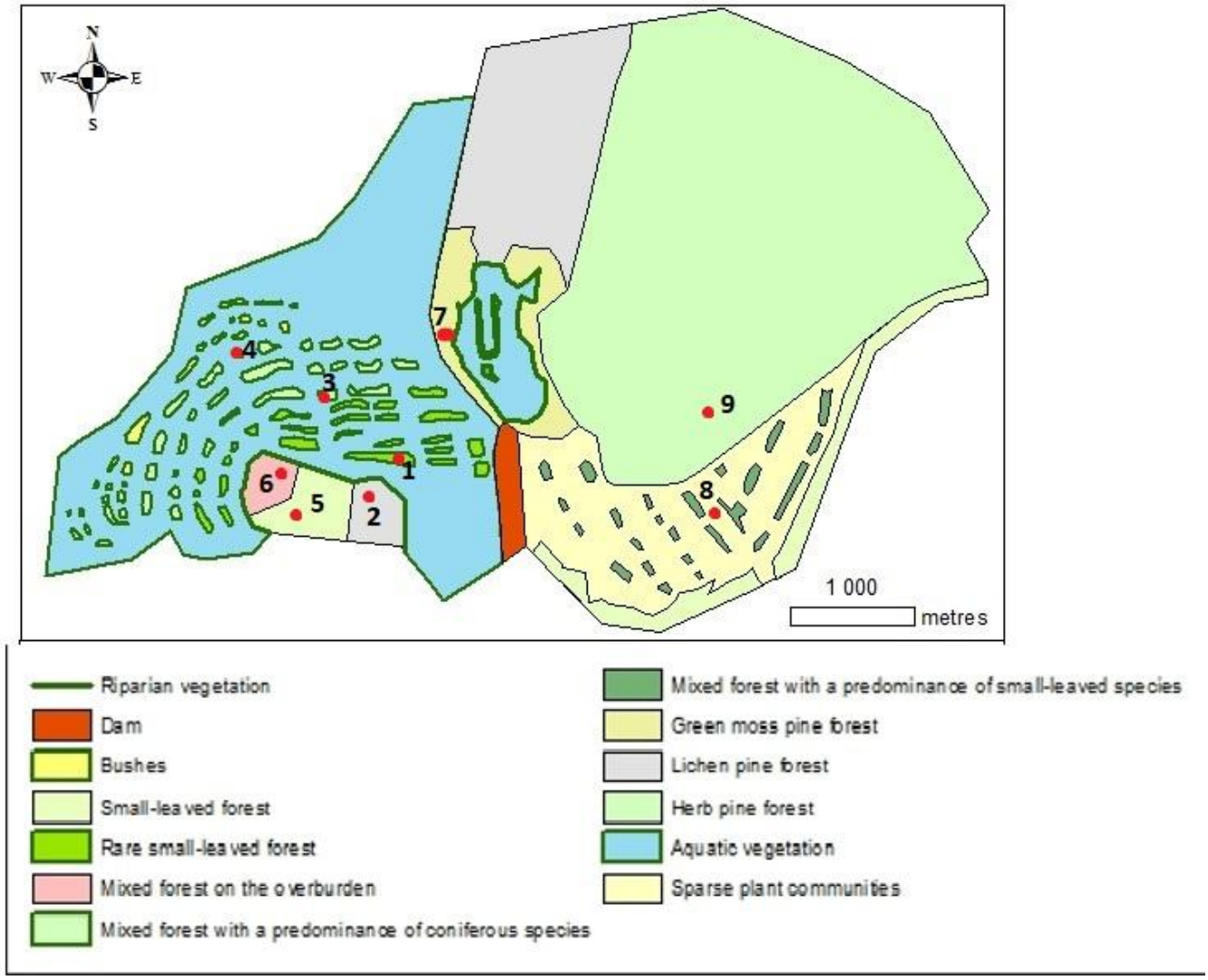
We conducted a lecture for children and local residents in the Slantsy Library, the main theses of which were printed on the official site of the Slantsy Children's Library.

Due to the fact that in the Slantsy it is planned to organize a large landfill to dump waste, local residents expressed concern about the sanitary condition of the area. Therefore, we have evaluated microbiological contamination of soils. According to the results obtained, the soil is clean.

Final conclusions

Thus, we described the soil microbiom of various parts of the Pechurki quarry. A distinct change in the species composition was observed on dumps of different ages during natural self-growth and reclamation. Based on the results obtained and the literature data, an improvement of the existing method of the reclamation is proposed. Serious work was carried out to involve local residents in the project and popularize the research results.

Annex 1. Figure 1. Schematic map of plant communities of the quarry and areas of study



Annex 1. Table 1. Descriptions of study sites

Sites	**Substrate**
Site 1. Self-overgrowing dump. Overgrowing duration - 28 years. Total projective cover of vegetation– 68 %.	**Grey-humus embryozem** **O** (0-4 cm) – litter, sandy loam, densely permeated by roots, dark, not greasy. **AY** (4-33 cm) – sandy loam, roots, washed quartz grains, lighter than previous. **C** (33-48 cm) – sandy, light, power plants spread out, the inclusion of clay particles and quartz grains, roots. **[C]** (48 cm) – consists of quartz grains and stones, large sand, darker than the previous one, a smooth transition (everywhere), fragments of limestone found.

Site 2. Self-overgrowing dump. Overgrowing duration - 16 years. Total projective cover of vegetation– 45 %.	**Abra(Al-Fe)humus embryozem** O (0-3 cm) - brown, turn rocks, quartz grains, a large number of roots, undecomposed pine litter. BF (3-13 cm) - the presence of roots, lighter previous, large rocks, wet, sand inclusions, quartz grains. C (13 cm) - stones, wet, gray, moving with streaks.
Site 3. Self-overgrowing dump. Overgrowing duration - 30 years. Total projective cover of vegetation– 56 %.	**Grey-humus hydrometamorphic embryozem** O (0-7 cm) - loamy sand, brown, lumpy structure, a large number of roots. AY (7-15 cm) - lighter than the previous, red streaks of oxidized iron, the presence of roots. Gox (15-46 cm) - lighter than the previous one, sand, roots, clay pellets. G (46 cm) - gray with accents of clay, iron concretions, rare roots, sand, decomposed organic residues.
Site 4. Self-overgrowing dump. Overgrowing duration -8 years. Total projective cover of vegetation– 60 %.	**Grey-humus replantozem** AY (0-26 cm) - sandy loam with inclusions of sand and clay, lumpy structure, incorporating stone, permeated with roots, ferruginous sand AC (26 cm) - the sand, lighter previous boundary layer along the rocky, ferruginous, permeated with roots, dark organic inclusions, a large number of stones.
Site 5. Accumulative self-overgrowing ecotope. Overgrowing duration - 35 years. Total projective cover of vegetation– 90 %.	**Grey-humus stratozem** O (0-13 cm) - badly decomposed woody debris, dark. AY (13- 25 cm) - the sand, the inclusion of stones, humus dark inclusions, the transition color, permeated with roots, minerals, grains. C1 (25-37 cm) - gray, sand, roots. C2ox (37 cm) - red sand with roots, ferruginous.
Site 5. Accumulative self-overgrowing ecotope. Overgrowing duration - 35 years. Total projective cover of vegetation– 90 %.	**Grey-humus stratozem** O (0-4 cm) - litter, decomposing organic debris, roots are present. AY (4-28 cm) - brown, a large number of roots, wood particles, loose, lumpy structure. C (28 cm) - sandy, light, moist, contains inclusions of iron root zone
Site 7. Recultivatioal plot of the quarry. Site 5. Overgrowing duration - 29 years. Total projective cover of vegetation– 57 %.	**Grey-humus replantozem** O (0-7 cm) - moss litter, poorly decomposed pine needles, half-decayed wood with plenty of mycelium. AY (7-9 cm) – humus-accumulative layer, gray, sand, a large number of roots. C (9+ cm) yellow, iron inclusions root zone, the inclusion of coal, a significant number of large stones.

Site 8. Self-overgrowing bottom of the quarry. Site 5. Overgrowing duration - 29 years. Total projective cover of vegetation– 10 %.	**Petrozem** C (0+ cm) - gray, with a lot of stones, very dense.
Site 9. Recultivatioal plot of the quarry. Overgrowing duration - 46 years. Total projective cover of vegetation– 50 %.	**Grey-humus replantozem** O (0-3 cm) - moss-hards, there are cereals. AU (3+ cm) loam, brown, turn rocks, roots, clay particles

To be kept and filled in at the end of your report

Project tags (select all appropriate):

This will be use to classify your project in the project archive (that is also available online)

Project focus: ✓ Biodiversity management ✓ Rehabilitation& habitat research ✓ Scientific research ✓ Soil management ✓ Species research Flora: Fauna:	Habitat: ✓ Soil Stakeholders: ✓ Authorities ✓ Local community ✓ Schools ✓ Universities