Research Article

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Microbiome of post-technogenic soils of quarries in the Republic of Bashkortostan (Russia)

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Abstract: The state of the microbial community is an essential factor determining the processes of soil restoration of disturbed soils. Quarrying and mining complexes are valuable sites for studying soil formation processes. Four different quarries (gypsum, sand–gravel mixture, sand, and clay) of the Republic of Bashkortostan were surveyed to determine the status of the microbial community of post-technogenic soils. Amplicon libraries of the 16s rDNA gene were analyzed. It was shown that the microbial communities of different soil horizons of quarries differ significantly. According to the results of the analysis, quarry communities demonstrate differences in the microbial composition of different horizons, while in some cases, the upper horizon is richer.

Keywords: 16s rDNA, quarries, sequencing, microbiome, 16s amplicon libraries

1 Introduction

One of the global issues related to ecology and soil science is land degradation (Graves et al. 2015; Gregory et al. 2015; Bagarello et al. 2018). Increasing

anthropogenic and technogenic impacts on land cover lead to its depletion and/or destruction (Misak et al. 2009). This entails the loss of valuable agricultural land and the degradation of natural landscapes, the restoration and reclamation of which are known to be very slow (Williamson et al. 1982; Pacheco et al. 2018; Gonzalez-Roglich et al. 2019). There is also a transformation in the natural functioning of landscapes, leading to a fundamental change in the provision of ecosystem services – reduced natural soil formation potential, increased risk of erosion, habitat degradation, and dramatic loss of biodiversity (Baude et al. 2019; Pena et al. 2020).

Consequently, it is necessary to restore these human-modified landscapes using methodological approaches that take into account key processes occurring at different spatial scales (Borda-Niño et al. 2017). For these purposes, the most effective method is to integrate all functions (biological, ecological, and socio-economic) of human-modified landscapes into a single whole landscape (Carmenta et al. 2020). Restoration also provides an opportunity to better understand the succession of land-based ecosystems in the human–environment interface to further manage the ecosystem services of landscapes (Ge et al. 2019).

Anthropogenic landscapes are in most cases transformed natural landscapes, whereas technogenic landscapes are landscapes formed with modified terrain elements (Semina and Androkhonov 2014). Newly developed elements of a heterogeneous terrain, in turn, become new niches for some species of flora and fauna (Dmitrakova et al. 2018).

Quarrying and mining complexes, which are anthropogenic landscapes, are valuable sites for the study of soil formation processes (Kimeklis et al. 2020). The soil cover is considered to be the basis of any terrestrial ecosystem, and therefore the speed of soil development in technogenic landscapes serves as an indicator of restoration and other ecosystem components. Soil microbiome is the main factor in formation processes,

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Figure 1: Location of research objects (No. 1–4 – sequential numbers of the studied quarries).

so its study remains a relevant task. One of the main methods of microbiome research is high-performance sequencing (Torsvik and Øvreås 2002; Soliman *et al.* 2017; Wei *et al.* 2018), which can be used to describe the microbial composition comprehensively and accurately (Janssen 2006). Analysis of the microbial composition of such soils indicates the rate of recovery and the nature of the processes taking place in them (Tripathi *et al.* 2017), which further provide a basis for subsequent land reclamation and use in agricultural activities (Abakumov *et al.* 2010, 2011).

During the development of quarries, mechanical destruction of the soil cover occurs primarily. This leads to a breakdown of all environmental factors and food chains that determine the formation of the microbial community in each specific soil. As a result, a primary zero substrate is formed. On these substrates in the future, the development of primary soil-forming processes begins in which the pioneering microbial community plays an important role. Thus, it is possible to determine which microbial community is the first to take part in primary soil-forming processes. In this context, our research aims to study the state of the microbial community in post-technogenic soils of different quarries using amplicon gene libraries of 16s rDNA.

2 Methods

The Republic of Bashkortostan is a geographically complex territory located in the southern part of the Ural Mountains within the limits of three physical and geographical units – the eastern part of the East-European Plain, the mountainous Southern Urals, and the sublime Trans-Urals, which explains the great variety of geomorphological and climatic conditions, soil-forming rocks, and vegetation types (Kadilnikov 1964; Khaziev 1995). Geologically, the Republic is located within two major tectonic structures – the eastern edge of the East-European Platform (Volga–Ural anticline – the elevated and plain Pre-Ural) and the western part of the Ural fold system (the Ural Mountains and the plain Trans-Urals) (Puchkov 2014).

This structure has predetermined a wide variety of rock constituents, causing the spread of various mineral deposits – oil, natural gas, coal, iron ore, copper, zinc, and gold, as well as construction materials (Fatkullin 1996). To date, the Republic of Bashkortostan has 946 deposits of construction materials (sand–gravel mixture, construction sand and stone, brick and expanded clay, gypsum, carbonate rocks for lime production, and agrochemical ores). Many quarries are not exploited;

they are abandoned, and no reclamation measures have been taken in them. The vegetation cover in them is restored exclusively by self-planting (Khabirova and Kulagin 2016; Kulagin and Habirowa 2016).

The research was conducted in July 2018. The following is a detailed physical and geographical description of the location areas of the quarries studied (Figure 1) and a morphological description of post-technogenic soils composing them (Table 1).

Quarry No. 1 is a gypsum mining quarry (N 54.483403, E 56.415787). It is located in the Iglinsky region of the Republic of Bashkortostan near the village of Okhlebinino, approximately 2 km south-east of the southern edge of the village. Along the high precipitous bank of the Belaya River, upstream after 1.4 km, flows the Sim river. The development of the quarry was completed about 12 years ago.

Quarry No. 2 is a sand and gravel mining quarry (N 54.587510, E 56.260058). It is also located in the Iglinsky region near the village of Karamaly, about 400 m northwest of the western edge of the village, in the steep southern slope of the hill. The development of the quarry was completed about 8 years ago.

Quarry No. 3 is a sand mining quarry (N 54.767979, E 55.836647). The quarry development was started in 2011. It is located in the Ufa region of the Republic of Bashkortostan near the village of Milovka, approximately 700 m south-east of the southern edge of the village. The territory of the sand quarry is located in the old bed of the Belaya River. The relief is leveled. The soil cover is represented by sandy fluvisol soil. The development of the quarry was completed about 5 years ago.

Quarry No. 4 is a clay mining quarry (N 54.833103, E 55.963042). It is located in the Ufa region of the Republic of Bashkortostan near the Alekseevka village, approximately 2 km northwest of the western edge of the village, in the second floodplain terrace of the Belaya River. Based on space data, it was found that the development of the first part of the quarry began in the early 2000s. The use of the second part of the quarry, from which samples were taken, began in 2013. Before that, there was arable land in the quarries. Soil 8A is the humus accumulative horizon of the virgin soil, which was removed before the development of the quarry and piled in a heap (in 2000). Part of the quarry was reclaimed (in 2018) by applying a humus-accumulative horizon to clay – that is 7AC, and part of the quarry was left without reclamation – 6AC.

According to the physical and geographical zonation of the Republic of Bashkortostan, the territories of gypsum and sand-gravel mixture quarries (quarries 1

and 2) are located in the Right Bank Pribelsky Physical and Geographical Region (subzone of the northern forest-steppe zone). The relief of this area is gently sloping with strong karst development and almost continuous development of broad-leaved forests on Greyic Phaeozem Albic soils. The most ancient formations coming out to the top surface are deposits of the Kungur tier of the Permian system, represented by gypsum, anhydrite, limestone, and dolomite. High sides of the valleys of the Belaya and Sim rivers are composed of gypsum, which caused the development of a gypsum quarry near the village of Okhlebinino.

Ufa (Upper Permian) red-colored rocks prevail in the western part of the district: sandstones, clays, marls with rare limestone interlayers, and local accumulations of pebble material and gypsum. Kazan (Upper Permian) sandy and clayey sediments are found in some areas. This is why extraction of sand-gravel mixtures was initiated near the village of Karamaly. Typical uplands in the county are marked by distinct asymmetries. The southern and western slopes are steep, separated by beams and ravines, and complicated by landslides and screes. The northern and eastern slopes are gentle and represent ancient surfaces of leveling.

The climate of the region is characterized by moderate continentality and medium moisture content. The average annual air temperature is 2.8°C. The average temperature in January is –14.3°C. Winter is characterized by stable frosty weather, snowfalls, and rare thaws. The average temperature in July is 19.3°C. Summers are warm and clear with sparse showers. The average annual rainfall is 450–500 mm (Kadilnikov 1964).

The territories of sand and clay quarries (quarries 3 and 4) are located in the Left Bank Pribelsky Physical and Geographical Region (subzone of the southern forest-steppe zone). The region is characterized by the development of extensive lowland terraced and gently sloping denudation plains, broad-leaved forests, and meadow steppes, as well as various forest-steppe soils.

Sedimentary rocks of the Permian, Tertiary, and Quaternary systems take part in the geological structure of the top surface. The Kungur sediments of the Permian period are represented by gypsum, and in the upper part of the thickness, they are interlaced with siltstones, marls, clays, sandstones, and limestones. The Kungur sediments are covered with rocks of the Ufa tier, represented by motley marls, clays, siltstones, sandstones, and limestones. On the slopes of river valleys and ravines, rocks of the Kazan Perm layer, limestone, sandstone, siltstone, and clay, are exposed. Quaternary sediments are represented in river valleys by alluvial

Table 1: Soil profiles and horizon characteristics

No.	Depth (cm)	pH H ₂ O	Corg (g kg ⁻¹ soil)	N alkaline hydrolyzable (mg kg ⁻¹ soil)	Description of soil horizon	Description of soil profile
Quarry No. 1 soil profile 1						
1W	0–2	7.6 ± 0.1	9.6 ± 0.3	27 ± 1.6	Gray-brown, medium loamy, friable, eluvium	Initial Rendzic Leptosols at the bottom of the quarry. Vegetation is very thin
1C	2–8	7.8 ± 0.2	2.1 ± 0.2	13 ± 1.2	Eluvium plaster	
Soil profile 2						
2AI	0–17	7.5 ± 0.1	12.4 ± 0.4	32 ± 2.3	Dark gray finely lumpy	Profile 2 is laid out in the same location, but is represented by more developed soil. Self-growing. Soil is Agro Sulfated Renzin
2C	18–22	7.7 ± 0.1	4.4 ± 0.2	16 ± 4.0	Brown, loamy eluvium gypsum	
Quarry No. 2 Soil profile 3						
3AU	0–23	7.4 ± 0.2	102.4 ± 7.8	150 ± 12.3	Dark, medium loamy, dense	Virgin soil is Rendzic Leptosols, forest-steppe (weed and grain grassland)
3CI	23–40	7.6 ± 0.1	23.6 ± 1.7	52 ± 4.2	Brown, covered by clayey coatings	
Soil profile 4						
4AC	0–2	7.7 ± 0.2	5.1 ± 0.2	15 ± 3.8	Sand–gravel mixture	Solid ground material heap without signs of soil formation at the bottom of the quarry. Self-growing
Quarry No. 3 soil profile 5						
5W	0–2	5.8 ± 0.2	10.3 ± 0.6	31 ± 2.2	Grayish, friable, loamy	The soil cover is represented by sandy fluvisol soil
Quarry No. 4 soil profile 6						
6AC	0–2	6.5 ± 0.1	42.4 ± 3.5	70 ± 6.0	Soil without reclamation	
Soil profile 7						
7AC	0–2	6.4 ± 0.1	36.8 ± 3.3	65 ± 5.4	Soil after reclamation from organo-mineral soil heap of Chernozemic genesis	
Soil profile 8						
8A	0–2	6.7 ± 0.2	73.5 ± 5.9	125 ± 10.3	Organo-mineral soil heap of Chernozemic genesis used for reclamation	

loams, sands, and gravels, and on watersheds and their slopes, by eluvial and diluvial loams and crushed rock. The main large relief elements in the region are the Belaya River valley and the watershed left-bank plain. The Belaya River valley reaches a width of 10–12 km. There is a floodplain, which is 5–7 m high and reaches a width of up to 5 km on the left bank. The floodplain is surrounded by coastal bars, old lakes, swampy karst, and suffosion depressions.

The territory of the clay quarry is located on the second floodplain terrace of the Belaya River. The relief is generally flat, and the slopes are insignificant, but there are numerous sinkholes of different diameters and depths. Absolute markings vary from 86 to 95 m. Soil-forming rocks are eluvial–diluvial carbonate clays and heavy loams. The soil cover is represented by Voronic Chernozem Pachic.

The climate of the region as a whole is continental with moderate humidification. The average annual air temperature is 2.8°C. The average temperature in January is 15°C. Winter is characterized by stable frosty weather, snowfalls, and rare thaws. The average temperature in July is 19°C. Summers are warm and clear with sparse showers. The average annual rainfall is 400–500 mm (Kadilnikov 1964).

From all soil profiles described above, three samples each weighing 100 g were taken for microbiological testing, and the depth of sampling is specified in the morphological description. DNA was isolated from 0.5 g of soil samples, using a DNA extraction kit MN NucleoSpin Soil Kit (MN, Germany), according to the manufacturer's protocol. Mechanical destruction of the sample was performed on a FastPrep homogenizer. The purified DNA was used as a matrix in PCR with universal primers for the variability section V4 gene 16S rRNA F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGA-CTACVSGGGTATCTAAT) (Caporaso et al. 2011).

Sample preparation and sequencing were performed on an Illumina MiSEQ (Illumina, USA) in line with the manufacturer's guidelines. Sequence quality filtration was performed in the Trimmomatic program (Bolger et al. 2014), and forward and backward readings were combined using fastq-join (Aronesty 2013). To search and filter chimeric sequences, vsearch was used (Rognes et al. 2016). OTU was determined using QIIME packages (Caporaso et al. 2010) by the method of Closed Reference, via the SILVA database (Yilmaz et al. 2014). OTUs attributed as chloroplastic/mitochondrial were removed. Alpha and beta analyses were also performed using the QIIME package.

Agrochemical analyses of soils were carried out using the methods reported by Sokolov (1975): the carbon content (Corg) was determined by using the Tyurin method with termination according to Orlov and Grindel; nitrogen alkaline hydrolysable (Nalk) was determined according to Cornfield; soil reaction (pH H₂O) was analyzed by potentiometry.

Data present in the Google Earth program, OpenStreetMap, and Yandex map service were used for spatial data analysis.

3 Results and discussion

An analysis of the morphological properties of the soils formed in the quarries shows that over the time since the development of quarries for the extraction of gypsum (quarry No. 1; ~12 years), sand–gravel mixture (quarry No. 2; ~8 years), and sand (quarry No. 3); ~5 years), as a result of the action of primary soil formation processes, a differentiation of the soil profile occurred. This resulted in the formation of a humus-accumulative horizon on the mineral substrate. In the clay quarry (quarry No. 4), the formation of the humus-accumulative horizon occurred as a result of reclamation (reclamation was carried out 2 years ago).

When considering samples taken from soil profile 1 (1W–1C, gypsum quarry No. 1), in the upper soil horizon 1W, the microbial complex is dominated by *Proteobacteria* and *Actinobacteria*, and it should be noted that they are also dominant in all the soils of the quarries studied below, which is generally characteristic of disturbed soils (Mummey et al. 2002; Li et al. 2014; Megharaj et al. 2017). Here, *Cyanobacteria* and *Patescibacteria* are also present (Figure 2), which is generally the case with gypsum soils (Cano-Díaz et al. 2018; Menéndez-Serra et al. 2019; Li et al. 2019). Alpha-diversity indices (Shannon and OTU) are naturally higher in the upper horizon (1W) (Figure 3). According to the beta-diversity data (Figure 4), these horizons are also dispersed.

In the lower 1C horizon samples from the same soil profile, a large number of *Actinobacteria* representatives are observed (Figure 2). Probably these large numbers of *Actinobacteria* representatives participate in the carbon and nitrogen cycle in natural ecosystems (Liu et al. 2017). It is due to the low permeability of gypsum and consequently the accumulation of nutrients and moisture in the eluvial column as a result of their supply

Figure 2: Community taxonomic composition.

Figure 3: Alpha biodiversity indexes: number of OTU (Observed – the number of “species” that are in the sample) and Shannon index (uniformity of representation of “species”). A larger index indicates a greater species diversity in the sample.

Figure 4: Beta biodiversity: PcoA weighted unifrac.

together with water, both from the 1W horizon above and from the height-dominant relief elements.

Samples of soil profile 2 (2AI–2C, gypsum quarry No. 1) show more developed soil and self-planting processes. These soil samples show a similar pattern of alpha diversity: the relatively high diversity in the upper 2AI horizon samples is replaced by low diversity in the 2C horizon samples (Figure 3). The lower 2C horizon compared to the upper 2AI is characterized by the presence of *Rokubacteria* and *Nitrospirae*. Previous studies show that *Rokubacteria* take part in the carbon cycle and are found on mineral substrates with a high content of available carbon (Becraft et al. 2017). The *Nitrospirae* perform important functions in the nitrogen cycle and prefer sulfate substrates (Lefèvre et al. 2011).

According to the beta diversity data microbiome of profile 1 (1W–1C), is different from soil profile 2 (2AI–2C) samples and are removed from each other (Figure 4). This is due to the presence of a large number of representatives of *Thaumarchaeota*, which is probably due to a higher level of self-planting of this area. The taxonomic composition of these samples is otherwise similar, except for a small amount of *Rokubacteria*, which is present in the lower layer of the section under study (Figure 2).

In quarry No. 2 (sand and gravel mining), soil profiles 3 and 4 were investigated. Soil profile 3 (3AI and 3CI) shows atypical alpha-diversity index ratios for soil

communities. Thus, samples from the upper 3AI horizon are relatively less diverse than the lower 3CI horizon (Figure 2). According to the beta-diversity data (Figure 4), the upper 3AI horizon is very different from the other samples (most likely due to the very high percentage of *Verrucomicrobians* in the community), which can be caused by the meadow vegetation type in the given area (Bergmann et al. 2011), as well as the optimal temperature and pH. The lower 3CI horizon is taxonomically (Figure 2) and in terms of beta-diversity (Figure 4) similar to the upper horizon of profile 1 (1W). This feature of the upper horizon may be related to similar microclimatic and soil-ecological conditions. These two points are located close to each other and belong to the same climate zone. The points are characterized by approximately the same water and thermal conditions. The upper horizons of profile 1 (1W) are mineral and have come to the surface only as a result of the development of a quarry. The lower horizon of profile 3 (3CI) is also mineral. These horizons are characterized by a low content of Corg and Nalk, as well as are slightly alkaline.

Soil profile 4 (4AC) differs in general in the average alpha diversity values considered (Figure 3). When considering the results of a weighted unifrac, these samples are grouped with samples 1W and 3CI (Figure 4). Taxonomically, these specimens are indeed very similar, except for the high proportion of *Firmicutes*

in the samples from Section 4 (Figure 2), which are found in the rhizosphere of plants and can survive extreme conditions (Zhang *et al.* 2019).

Section 4 is the lower part of the gentle slope. Dissolved organic substances and nutrients from top soils due to groundwater are included here. These dissolved nutrients at the bottom of the quarry contribute to active self-planting and a high proportion of representatives of the Firmicutes phylum.

Samples from section 5W (quarry No. 3, sand mining) also show unusually high alpha-diversity values for anthropogenically disturbed soils (Figure 3), which is probably due to their sandy particle size distribution, their location within the old riverbed, and the corresponding water and nutrient regimes. Beta-diversity metrics bring these samples closer to the cluster 1W–3CI–4AC, even though at some distance (Figure 4). Taxonomic analysis did not reveal any significant differences from the typical soil profile, except for a slightly larger share of *Thaumarchaeota* (Figure 2). *Thaumarchaeota* oxidize ammonia and play an important role in biogeochemical cycles such as the nitrogen cycle and the carbon cycle. At the same time, *Thaumarchaeota* are adapted to conditions with a low level of available nutrients and oxygen (Schleper and Nicol 2010), which is typical for the floodplain conditions of the sand quarry located in the floodplain of the Belaya River.

Finally, when considering the reclamation system (clay quarry No. 4), three soil profiles were analyzed: 6AC – soil without reclamation, 7AC – soil after reclamation, and 8A – soil for reclamation. The original soil of heap (6AC) is characterized by extremely low alpha diversity indices (Figure 3), which may indicate low intensity of soil formation. Also, the original soil is very different according to the beta-diversity data (Figure 4). The reason for this is the large number of *Verrucomicrobia* representatives, as well as a decrease in the proportion of *Proteobacteria* and *Bacteroidetes* and the absence of *Thaumarchaeota* in comparison with the communities of soil samples 7AC and 8A (Figure 2). Sample 7AC – soil after reclamation – and sample 8A – soil for reclamation – are similar: they show high alpha index values (Figure 3) (interestingly, they are somewhat lower in the original soil), and according to the beta-diversity data, they are similar and grouped with samples from soil profile 2 (2C) (Figure 4). Taxonomically, they contain many representatives of *Thaumarchaeota* (which, most likely, explains clustering with samples of Section 2).

4 Conclusions

Thus, the estimation of the condition of the microbial community formed during primary soil formation processes in the substrates formed as a result of the development of quarries for the production of gypsum, sand and gravel, sand, and clay located within the sloping foothill plains of the Southern Urals (Russia, the Republic of Bashkortostan) under the conditions of a forest-steppe climatic zone has been made. The assessment of the microbial community was carried out using amplicon gene libraries of 16s rDNA. Studies have shown that primary soil formation processes occur in all quarries; the formation of the upper humus-accumulating horizon on the underlying mineral substrate is observed, and self-growth processes actively take place. The microbial communities of the quarried soil profiles are dominated by carbon and nitrogen cycle species *Cyanobacteria* and *Patescibacteria*, which is characteristic of disturbed soils. They are characterized by the dominant alpha and beta indexes. The species diversity of microbial communities in the mineral horizons is influenced by their chemical and particle size distribution, water and nutrient regimes, and extreme environmental conditions.

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