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Microbiome composition of disturbed soils from sandy-gravel mining complexes with different reclamation approach

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Abstract

Activities connected to mineral mining disrupt the soil layer and bring up to surface parent rock material. As a result, they leave behind vast areas of disturbed lands, that are difficult to restore due to altered environmental conditions. Returning these lands to the natural ecosystems is an important contemporary challenge. Soil microbiome composition reflects changes happening to disturbed lands, its analysis helps to evaluate disturbance degree and estimate the effect of implementation of remediation techniques. Also, factors connected to the characteristics of a particular geographical region have a certain impact and should be taken into account. We focus on microbiomes of disturbed lands from two sandy-gravel mining complexes in mountainous areas with moderate continental climate (Central Caucasus, Russia). These quarries share the same parent rock material but differ in benchmark soil type and presence of remediation practices. Comparative analysis of microbiome composition based on sequencing of 16S rRNA gene libraries showed that region and disturbance are the key factors explaining microbiome variation, which surpass the influence of local vegetation factors. However, application of remediation techniques greatly reduces dissimilarity of soil microbiomes caused by disturbance. Linking of soil agrochemical parameters to microbiome composition showed that disturbance factor correlates with a lack of organic carbon. Other agrochemical parameters, like pH, ammonium, nitrates and total carbon explain variation of microbiomes on a smaller scale between sampling sites. Thus, while regional and disturbance factors reflected differentiation of soil microbiomes, soil agrochemical parameters explained local variation of certain groups of microorganisms.

Keywords

16S rRNA, amplicon library sequencing, primary soil, open-pit mining, quarry, remediation techniques, soil microbiome

Introduction

One of the global ecology and soil science problems is land degradation (Jie et al. 2002, Gregory et al. 2015, Právělie 2021). The industry grows faster every year, which in turn changes the natural ecosystems. Minerals are extracted; by mining, open-pit, and combined methods. Open-pit (quarry) extraction is the cheapest and therefore prevails (Abakumov and Gagarina 2006). Open-pit mining causes the greatest damage to the landforms (Chen et al. 2015). For example, open-pit mining in forested areas is associated with cutting down trees, draining ponds, and rivers, and streams are diverted beyond the deposits. Negative changes occur not only at the extraction sites but also in adjacent territories. The areas affected by open-pit mining are much larger than the quarry area (Bekarevich et al. 1969, Melnikov 1977, Monjezi et al. 2008). Open-pit mining results in the formation of dumps which can serve as an example of a negative human impact on the ecosystem (Burlakovs et al. 2017, Puell Ortiz 2017). This negative impact can be eliminated by implementation of mine reclamation techniques. These include diverse practices – restoration, rehabilitation, or replacement - aimed at returning disturbed lands to the natural ecosystem by restoring or giving them new function (Bradshaw 1984, Favas et al. 2018). Choice of reclamation approach depends on the available resources and tasks. In case of open pit mining the important task is to remove dumps and restore the surface level. It can be achieved by backfilling the quarry pit with the dumps and overburden material (Jurek 2014, Legwaila et al. 2015). In cases when no techniques are applied to abandoned mines due to economical or other difficulties, they can undergo passive recovery with consequent spontaneous vegetation (Holl 2002, Prach et al. 2013).

The effect of degraded land transformations can be accessed by analysis of agrochemical and biological soil properties (Gavrilenko et al. 2011, Murugan et al. 2014, Gorobtsova et al. 2016, Kazeev et al. 2020). Studies of soil microbial biomass and microbe enzyme activity have shown that soil microbiota is the first to respond to changes in the soil (Józefowska et al. 2016). Nowadays, high-throughput sequencing of 16S rRNA gene libraries becomes fast and effective tool for gathering huge amounts of genetic information, which becomes more effective in characterizing changes in soil microbial communities. Although there are many studies connecting effects of different types of agricultural practices on soil microbe communities (Coller et al. 2019, Chen et al. 2020, Liu et al. 2021), such studies of soils disturbed by mining are scarce (Epelde et al. 2014, Sun et al. 2019). Comparative analysis of soil microbiome can reveal relationships between its composition, soil disturbance factor and agrochemical parameters (Liddicoat et al. 2019). For example, it was shown that microbiomes of technically reclaimed coal mines differ by bacterial abundance and diversity from natural soil, but with time their diversity evens out (Hou et al.

2018). Furthermore, some of the top bacterial taxa can be linked to agrochemical and consequent functional changes in disturbed land.

There are huge areas of degraded soil in the Central Caucasus regions. As specified in the government statement (Abramchenko et al. 2019), the degraded soil area in Stavropol Krai is 3,400 ha, and 1,007 ha in the Kabardino-Balkarian Republic. The dominating activity that leads to land disturbance in the region is the extraction of common minerals such as boulder-sand-gravel mixes, construction sand, building stone, clays, etc. We managed to find two boulder-sand-gravel mining complexes on different soil types with different reclamation techniques applied. Considering the above, the aim of the research was to link microbiome composition of soils formed on the open-pit dumps and benchmark soils of adjacent Central Caucasus territories (Stavropol Krai and the Kabardino-Balkarian Republic) with disturbance, reclamation, region, and agrochemical composition factors.

Material and methods

Sampling sites were located in the foothills of the Central Caucasus in two regions – Urvan (Kabardino-Balkarian Republic, Russia) and Progress (Stavropol Krai, Russia) (Fig. 1). The terrain of both study areas can be characterized as hilly plains. According to the classification (Sokolov and Tembotov 1989), they belong to the belt of meadow steppes (400-800 m above sea level) of the Elbrus variant of the zonation (Progress) and the steppe zone (200-400 m above sea level) of the Terek variant zonation (Urvan) of the Central Caucasus. In the studied territories, the climate is moderately continental, with a long frost-free period, hot summers, and little snow, with frequent thaws in winter. In the zone of meadow steppes (Progress), the average annual precipitation is 579 mm/year, the average annual air temperature is 10.45° C, the total evaporation is 864 mm/year (Razumov et al. 2003). In the steppe zone (Urvan), the average annual precipitation is 522 mm/year, the average annual air temperature is 11° C, the total evaporation is 818 mm/year (Ashabokov et al. 2005). Two regions have different soil types and water regimes: Phaeozems in Progress were formed under the influence of only atmospheric moisture, with a periodic leaching regime, while in Urvan Umbric Gleyic Soils are characterized by increased surface watering and additional film-capillary moisture, the source of which is shallow (1.5-3 m) located groundwater. The bluish-gray inhomogeneous coloration of the lower horizons of meadow soils is a weakly pronounced sign of hydromorphism (Duchaufour 1982).

In each region we found an abandoned territory of a quarry located on a deposit of sand and gravel mixture. Both territories consist of multiple differently aged pits and rock dumps, but with different soil types. The first mining site was found in Urvan' district of Kabardino-Balkaria near the terrace of the eponymous river, which flows between two quarry pits. The deposits in this area have been developed since 1958. Benchmark soil type for this area is Umbric Gleyic Soil, which remains undisturbed near the riverbanks. Abandoned quarry pits showed signs of passive recovery with spontaneous overgrowth by *Populus*, *Hippophae* and reed. The second mining site was found in Kirovsky district of Stavropol Krai near Malka River. The field has been developed since 2000s. The flat lands surrounding the

quarry complex belong to Phaeozem soil type which are completely converted for farming purposes. Thus, the nearest benchmark soil for this territory is an Agrisol. Of course, Agrisol itself is a disturbed soil (Conacher 2009, Lupatini et al. 2017, Wipf et al. 2021), but in this case, we treat it as a benchmark soil, due to lack of native soil nearby. Technical reclamation, consisting of backfilling the bottoms of the abandoned pits with a mixture of overburden Phaeozem, sand and gravel, was implemented in this area. Vegetation in the quarry pits varied from Ambrosia to Acacia thickets.

Sampling sites were picked so that they would represent soil native to this area along with disturbed soils in different stages of overgrowth, which was determined by sight on the spot and confirmed partly by satellite images from different years (Suppl. material 1, Figure S1). At the Urvan region we collected samples in the two neighboring quarry pits: one fully abandoned at the time of collecting, the other partly functional, and benchmark soil near the river (Suppl. material 1, Figure S2a). At the Progress region we collected samples in the old overgrown quarry pit currently used for pasture, then in the newly excavated and freshly overgrown two-year mining pit, and Agrisol from the nearest field, where the crops (corn) have already been harvested (Suppl. material 1, Figure S2b). More detailed information is presented in Table S1 (Suppl. material 2). For each site we took 2-4 biological replicates from slightly different ecological microniches, e.g., in Urvan region the quarry bottom had heterogeneous distribution of vegetation, varying from moss and grass cover to thickets of *Populus*, while benchmark soil samples varied in the burnout degree of the meadow due to different distance from the river. In the Progress region two quarry pits varied in age (approximately 2 vs 10 years), grazing factor (present in the older pit) and vegetation (grass cover vs *Acacia* thickets), benchmark soil samples were taken from one field, but before and after the rain. Thus, we set out to investigate the possible differences between these replicates. At each sampling site we made a soil cut, measured temperature in the top 2-5 cm layer using a digital thermometer. From the same top layer soil samples were collected in plastic tubes with subsequent same day freezing at -20°C for molecular analysis and into plastic 1 L bags with subsequent air drying for agrochemical analysis.

For all dried soil samples agrochemical analysis was performed, including measuring of pH, organic carbon (OC), ammonium (NH_4^+), nitrate (NO_3^-), mobile phosphorus (P_2O_5) and potassium (K_2O), as previously described in Gladkov et al. (2019). Total carbon content (TC) was determined by direct combustion on the elemental analyzer Euro-EA3028-HT (Evrovector, Italy) at the St. Petersburg University Research Park. ANOVA with Tukey HSD test, t-test group comparisons and correlation coefficients of the results were calculated in Statistica 13 (TIBCO Software Inc., USA).

From each sample of the frozen soil total DNA was extracted in quadruplicate, and consequently used for the construction and sequencing of the 16S rRNA amplicon libraries using Illumina MiSeq (Illumina, Inc., USA) as described in Gladkov et al. (2019) at the Centre for Genomic Technologies, Proteomics and Cell Biology (ARRIAM, Russia). Obtained data was processed and visualized as described in Kimeklis et al. (2021) in R (R Core Team 2021) and QIIME2 (Bolyen et al. 2019) software environments using the following tools: dada2 (Nearing et al. 2018), RDP Classifier with 50% confidence threshold (Wang et al. 2007), SILVA Release 138 (Quast et al. 2013), phyloseq (McMurdie and

Holmes 2013), DESeq2 (Love et al. 2014), vegan 2.5-7 (Oksanen et al. 2020), ggpubr 0.4.0 (Kassambara 2019), picante (Kembel et al. 2010), ggforce 0.3.3 (Pedersen 2019), tidyverse (Wickham et al. 2019), ggtree (Yu et al. 2018), ampvis2 (Andersen et al. 2018) in RStudio (RStudio Team 2020) and SEPP package (Janssen et al. 2018). Analysis of compositional microbiota data (balances) was performed by PhILR transformation (Silverman et al. 2017). The code is available in the supplement (Suppl. material 3).

Results

Sampling

In total we collected 21 soil samples at 7 sites from 2 regions (Table 1). Samples N1-N3 were from Urvan and samples N4-N7 from Progress. N2 and N4 samples were taken from benchmark soil, others - from disturbed soil. These samples were collected during several days with differing weather conditions: during the first day at the Urvan' quarry complex it was sunny and dry, but on the following day at the Progress quarry it was cooler and started raining. We waited until more favorable weather conditions arose and returned the next day to collect samples. Thus, for the second region we have benchmark soil samples taken before (N4-1) and after (N4-2) the rain, but all other samples were taken the next day after the rain.

Soil agrochemical parameters

Agrochemical parameters of studied soils varied between sites and samples, with different parameters changing with different factors (Table 1). ANOVA with post-hoc Tukey HSD test showed that region factor significantly affects soil temperature, pH, TC, potassium, ammonium, and nitrates. Disturbance factor (between benchmark and primary soil samples) affected OC and pH. Soil pH in Urvan region was alkaline (7,4-8) and didn't show significant difference between quarry (N1 and N3) and benchmark (N2) sites ($t = 1,09$, $p = 0,33$) (Table S2). Soil pH in Progress region was slightly alkaline, ranging between 6,9 and 7,6, with quarry sites (N5-N7) being more alkaline (7,2-7,6) than benchmark N4 soil (6,9-7,1) ($t = 4,52$, $p < 0,01$). OC quantities ranged from low to very low and had significant differences between quarry (0,2-0,8%) and benchmark (1,8-2,7%) sites for both regions ($t = -14,60$, $p < 0,01$). Some factors had significant correlation between each other (Table S3): phosphorus and ammonium ($R^2 = 0,73$, $p < 0,05$), ammonium and nitrates ($R^2 = -0,53$, $p < 0,05$), pH and nitrates ($R^2 = -0,61$, $p < 0,05$), phosphorus and nitrates ($R^2 = -0,52$, $p < 0,05$), phosphorus and potassium ($R^2 = 0,48$, $p < 0,05$). Phosphorus content variation couldn't be attributed to region or disturbance factor. Ammonium content was higher than nitrates in all sites, except N4 (benchmark Agrisol) and N5 (2-year self-growing quarry with fresh dumps of soil and rock mixture), which is in very close to N4. Both N4 and N5 samples stood out from the rest as they had the smallest amount of phosphorus, ammonium, and the maximum of nitrates. Interestingly, N4 soil samples taken before and after the rain didn't differ significantly in any parameters, except nitrates, which increased twofold after the rain. Samples from sites N6 and N7 from the same quarry bottom had the highest potassium content among all samples. To conclude, while pH, TC, ammonium,

nitrate and potassium values demonstrated region specificity, OC values were associated with disturbance factor, being higher in benchmark soils compared to primary soils of quarries in both regions.

Sequencing data processing

Total of 84 libraries of 16S rRNA gene were sequenced, resulting in 1768209 reads, which split into 10976 amplicon sequence variants (ASVs). Minimum reads per library was 7397, maximum 36229, median - 20948, mean - 21050. Reads not assigned on the phylum level (0,09% of total count) were deleted from the dataset. From the other reads 96,77% were attributed to class, 87,29% - to order, 70,03% - to family, 40,02% - to genus and 2,11% - to species. Urvan region showed 6113 unique ASV, Progress - 3238, and 1625 (corresponding to 65,3% of all reads) were common (Table 2). In Urvan 1917 unique ASV were detected in benchmark, 5100 - in quarry, 711 (55,9% of reads) were common. For Progress there were 534 unique ASV in benchmark, 3265 in quarry and 1064 (85,7%) in common. The datasets generated and analyzed for this study can be found in the BioProject Database (<https://www.ncbi.nlm.nih.gov/bioproject/>) via ID PRJNA777426.

Alpha and beta diversity analysis

Alpha diversity was accessed by 4 indexes - Observed, PD (Faith 1992), Shannon (Shannon and Weaver 1949), and inverted Simpson (Simpson 1949) (Suppl. material 1, Figure S3). Values of these indexes had little variation, but some significant differences were observed. At first, for each region we detected significant differences between benchmark and disturbed samples, but only for inverted Simpson index, which represents the probability that two randomly picked sequences belong to different ASVs (Fig. 2). In both cases it was higher for quarry sites than benchmark. Then, in Urvan all indexes for samples from all sites differed significantly between each other with no relation to disturbance factor, with N3 site being the most diverse and N1 – the least. Apart from that, dispersion of most alpha indexes of samples from Urvan was higher than in Progress. Indexes of alpha diversity allow us to estimate microbiome variation within samples, and in our case, we can assume that microbiome variation from Progress is more consistent across different sites and biological replicates, while from Urvan it is more diversified.

Beta diversity was calculated using Bray-Curtis distance matrix (Bray and Curtis 1957) and visualized by NMDS (Kruskal 1964) (Fig. 3). Unlike alpha-diversity, beta-diversity revealed differences between samples in a more defined manner. PERMANOVA (Anderson 2017) performed with adonis2 test showed that microbiomes of quarry and benchmark samples differ significantly for both regions - with R^2 - 0.26 (p -value = 0.001) for Urvan, and R^2 - 0.11 (p -value - 0.002) for Progress. It's values also show that disturbance is a greater factor of explained variability in microbiomes for the soils in Urvan than in Progress. Data visualized by NMDS matches with PERMANOVA, as we can see 3 distinct groups: (1) Urvan quarry samples N1-N3, (2) Urvan benchmark N2 and (3) all Progress samples N4-N7.

For the Urvan region samples from both quarry pits group closer together, while benchmark samples are separated from them. On the other hand, for the Progress region separation of microbiomes of benchmark and disturbed soils is less apparent.

Apart from differences between sites, we also investigated differences between biological replicates within one site. Analysis of multivariate homogeneity of group dispersions (Anderson 2005, Anderson et al. 2006) showed significantly higher distance to centroids values between replicates at Urvan sites (ANOVA p-value < 0,001) than for Progress sites, despite the similar level of differences in ecological microniches (Suppl. material 1, Figure S4).

Phylogeny composition

The most abundant phyla across all samples were typical of soil microbiomes - Actinobacteriota, Acidobacteriota, Alpha- and Gamma- proteobacteria, Bacteroidota, Crenarchaeota, Firmicutes, Verrucomicrobiota, Planctomycetota, Chloroflexi, Myxococcota and Gemmatomonadota (Fig. 4). According to the heatmap, relative abundance of phyla mirrors differences between samples like beta-diversity: benchmark (N2) and quarry (N1, N3) samples from Urvan site demonstrate difference in quantities of phyla Bacteroidota, Crenarchaeota, Firmicutes, RCP2-54, Patescibacteria and Entheonellaeota, while for Progress samples there is no evident difference in major phyla composition between benchmark (N4) and quarry samples (N5-N7). As for location-specific phyla, in Urvan Acidobacteriota, Planctomycetota, Chloroflexi show higher relative abundance, while in Progress - Verrucomicrobiota.

On the family level we still see that major groups are present in all samples, but their abundance differs between samples (Suppl. material 1, Fig S5). Top taxa are Nitrososphaeraceae and Planococcaceae (higher values at benchmark sites), Chitinophagaceae (higher values at quarry sites), Pyrinomonadaceae, Sphingomonadaceae and Chtoniobacteriaceae. Apart from these, Urvan site has variation in the content of the following families between benchmark and quarry samples: Pseudonocardiaceae, Micromonosporaceae, Beierinckiaceae, Bryobacteriaceae, Commamonadaceae are more prevalent in quarries N1 and N3, while Propionibacteriaceae - in benchmark N2. For Progress distribution of families doesn't seem to be linked to quarry/benchmark distinction, but rather to different quarry sites.

Shifts in ASVs abundance

Statistically significant shifts on the genus level between regions and disturbance factor were accessed by differential abundance (DA) in DESeq2 (Fig. 5). Only ASVs with basemean 10 or more are left in the analysis, with adjusted p-value < 0,05. With this cutoff Urvan has 45 more abundant ASVs, Progress – 164 (Suppl. material 2, Table S4). The highest modulo values of log2FoldChange are detected in the ASVs with basemean < 100. ASVs with basemean values exceeding 100 have smaller log2FoldChange values, meaning they are present in both regions, but most of them are prevalent in the Progress. No apparent phylum tends to be more characteristic of any region. Both regions have

different prevalent ASVs from Acidobacteria, Actinobacteria, Bacteroidota, Crenarchaeota, Gemmatimonadota, Proteobacteria and others.

Comparisons between benchmark/quarry samples show different dot distribution in two regions. In Urvan there are 37 ASVs more prevalent in the benchmark samples and 106 ASVs in the quarry (Suppl. material 2, Table S5). The most abundant ASVs in Urvan benchmark site belong to Acidobacteriota (5), Actinobacteriota (6), Firmicutes (11) and Crenarchaeota (7). Top phyla of ASVs from quarry were Acidobacteriota (24), Actinobacteriota (31), Bacteroidota (13), Proteobacteria (19) and Crenarchaeota (6). In Progress 4 ASVs were more abundant in benchmark soil microbiomes, all of which belong to Crenarchaeota; while 33 ASVs were detected as more abundant in the quarry microbiomes, most of them belonging to Acidobacteriota (3), Actinobacteriota (10), Bacteroidota (8), Proteobacteria (7) (Suppl. material 2, Table S6).

Several trends could be highlighted from the DA analysis data. ASV shifts are the most contrasting between regions. Within regions contrast between quarry and benchmark is more pronounced in the Urvan, than in Progress. Quarry microbiomes of both Urvan and Progress regions have a larger proportion of minor ASVs in comparison to benchmark microbiomes. In all comparisons ASVs with higher basemean values had lower modulo values of Log2FoldChange, while ASVs with small basemeans have higher modulo values of Log2FoldChange.

Links between taxonomic composition and soil agrochemical soil properties

Canonical Correlation Analysis (CCA) (ter Braak 1986, Palmer 1993, McCune 1997) was used to link beta-diversity of microbiomes with soil agrochemical properties. Biological replicates of microbiomes from different sites allowed us to build a significant model (ANOVA p-value = 0,002). For this analysis temperature data was scaled to the deviation from the day's mean. Most significant factors were pH (p-value = 0,003), OC (p-value = 0,009), ammonium (p-value = 0,006) and scaled temperature (p-value = 0,032) (Suppl. material 2, Table S7). According to the Variance inflation factors (VIF) test (Fox and Monette 1992, Fox 1997), these factors were not multicollinear, meaning their influence on samples' microbiomes was independent (Suppl. material 2, Table S7). Data is visualized on the CCA plot, where arrows point the direction of factors influence, and dots represent microbiomes (Fig. 6a). The farther the dot is relative to the direction of the arrow, the more factors explain variation. If the dot is in the opposite direction, then the factor influences negatively on this ASV. In Urvan microbiomes of quarry sites N1 and N3 are mostly affected by pH and ammonium, while those of benchmark N2 site are mostly affected by OC. All microbiomes from Progress site are influenced by nitrates and TC quantities. Progress samples show less dependence on agrochemical factors than Urvan, because they are grouped closer to each other and are closer to the 0,0 point. On the other hand, Urvan samples are quite dispersed far from 0,0. In accordance with beta-diversity, microbiomes from benchmark and quarry samples from Urvan (N1-N3) exhibit more pronounced difference between each other, which can be linked to influence of agrochemical parameters, than samples from Progress (N4-N7), which group more closely to each other.

CCA plot on the Fig. 6b shows how singular ASVs in this dataset are affected by agrochemical factors. Majority of the top 100 abundant ASVs are dispersed in the direction of OC and nitrates. These include ASVs from different phyla, the most reactive ones belong to *Bacillus*, Nitrososphaeraceae, *Microlunatus*, *Acidibacter*, *Xiphinematobacter*, *Nitrospira*, Gaiellales. Some of these ASV match those which are statistically more prevalent in benchmark sites (Table S5 and S6). In the opposite direction of OC there are ASVs, associated with the lack of OC. These are RB41, *Ramlibacter*, *Flavisolibacter*, *Asanoa*, *Puia*, *Niastella*, *Briobacter*. Some of these ASVs are also detected as characteristic of quarry microbiomes by Log2FoldChange analysis (Suppl. material 2, Table S5 and S6). Other factors influence microbiome composition, which could be linked to some other differences between samples, not linked to disturbance or region factor. Such factors include ammonium and nitrates: they influence the microbiome composition in opposite directions, so that ASVs reacting to the presence on ammonium (*Pseudonocardia*, *Solirubrobacter*, *Acidibacter*) and nitrates (*Udaeobacter*, Nitrososphaeraceae, Gaiellales) could be distinguished.

We also used CCA to show connection between microscale (agrochemical parameters) and macroscale (region, disturbance) factors and their influence on microbiome composition. If we put on CCA plot only ASVs, significantly changing between regions (detected by DESeq, Suppl. material 2, Table S4), we see that ASVs split in two groups: the ones from Urvan are on the side of ammonium and pH influence while on the opposite side there are ASVs from Progress region, which are more influenced by TC and nitrates. Both regions have characteristic ASVs from Acidobacteriota, Actinobacteriota, Proteobacteria, Crenarchaeota and other phyla.

Figure S6b (Suppl. material 1) demonstrates CCA plot with ASV which were revealed as statistically different between quarry and benchmark samples for each region by DESeq analysis (Suppl. material 2, Table S5 and S6). Here ASVs located on opposite sides of OC, which is consistent with the fact of correlation between OC quantities and soil disturbance. These ASVs are mostly from Acidobacteria, Proteobacteria, Actinobacteria. Groups corresponding to presence of OC (Crenarchaeota, Firmicutes) are divided by two: one between OC and ammonium, corresponding to ASVs from Urvan benchmark, and the other between OC and nitrates, corresponding to Progress benchmark. Notable, ASVs with the same taxonomy (Nitrososphaeraceae, Vicinabacteriaceae, Gemmatinomonadaceae) can be seen in the groups correlating with both presence and absence of OC.

Phylogenetic compositional analysis

Analysis of 16S rRNA gene libraries is often based on negative binomial distribution, which have some limitations. These methods make a Type I error in assessing changes in the microbial community at high taxonomic levels (Lin and Peddada 2020, Nearing et al. 2022). PhILR transformation offers an approach to overcome statistical artifacts of relative abundance of microbiota and analyze compositional data. It reveals “balances” which allows to further investigate into relating of phylogenetically close microorganisms to different factors (Suppl. material 1, Figure S7). Nine significant balances for Urvan and seven significant balances for Progress were identified. It was shown that for Progress

significant differences are at low taxonomic levels (differences in individual ASVs within genus or family) (Figure S7A). In contrast, for Urvan, several balances (n210, n733, n788) show differences at phyla-class levels (Figure S7B). The response is especially diverse at different taxonomic levels within the Acidobacteriota phyla. Differences in ASV content within the phyla Firmicutes, Chloroflexi, and Verrucomicrobiota is also shown in the quarry sites.

Discussion

Linking to previous studies

This work is a continuation of the research about the microbiomes of soils from different climate zones, recovering from anthropological damage, primarily mining of parent rock material (Gladkov et al. 2019, Ivanova et al. 2020, Pershina et al. 2020, Zverev et al. 2020, Abakumov et al. 2021, Kimeklis et al. 2021). Here we explore the area between moderate continental and subtropical zones, located in the northern foothills of Caucasus mountains. The specificity of this study is that we collected samples from the same type of sandy-gravel quarries in close regions (approximately 50 km) with different benchmark soil types. The key difference between sampling sites was that quarry pits at one region (Progress) were reclaimed by backfilling using soil heaps from the overburden Agrisol, while the other (Urvan) were left to passively recover with spontaneous vegetation overgrowth on parent rock material. So, we were able to analyze different patterns of microbiome restoration on similar parent rock material in one climatic zone, but different benchmark soil types and applied reclamation practices.

Profiles of weakly developed soils of quarries usually consist of two horizons: W - accumulated humic material, and C - parent rock underneath, usually the overburden material (Abakumov 2008). In our previous studies we compared microbiomes of these horizons within and between sampling sites and it turned out that microbiome of parent material is the reflection of topsoil horizon and that between sites their microbiomes shift simultaneously (Kimeklis et al. 2021). Putting this into consideration, in this study we limited ourselves to the top horizon of each site.

In our previous studies from the quarries of northern regions we observed that primary soils of quarries are colonized by photosynthetic bacteria – Cyanobacteria and Chloroflexi (Gladkov et al. 2019, Kimeklis et al. 2021). These microorganisms form biofilms or soil crusts and can successfully colonize substrates deprived of organic carbon resources (Malard and Pearce 2018), but in this work presence of these groups of microorganisms was minuscule. Perhaps, it can be explained by overall lower humidity of the southern region and warm arid conditions in the period of sample collecting.

Factors influencing microbiome composition

Factors which influence microbiome composition can be put into hierarchical categories based on their complexity and scale of effect (Deakin et al. 2018). Large-scale differences, like regions, distance, or type of agricultural practice, usually are considered the main

factors of microbiome variation (O'Brien et al. 2016, Deakin et al. 2018, Shi et al. 2018). On the other hand, local overgrown vegetation, which is one of the bases of humic layer accumulation (Abakumov et al. 2020), usually creates spatial variations, which translate into micro-scale differences in microbiomes (Schreiter et al. 2014, Mitter et al. 2017). The same goes for comparisons between seasons and distance - while seasons create variation due to fast-changing factors, geographical differences explain more of microbial variation (Zhang et al. 2020, Wang et al. 2021). In our study we detected differences between biological replications caused by micro-scale factors, like vegetation, insolation, or water regime, but they didn't overcome diversity created by large-scale factors - region and disturbance. There is evidence that application of soil reclamation techniques shortens recovery period and stabilizes microbial community (Hou et al. 2018). Here we detected the same effect: application of backfilling in the quarries of Progress region led to greatly reducing of microbiome dispersion and distinction between disturbed and benchmark sites. On the other hand, quarry microbiomes of Urvan region demonstrated higher distinction from benchmark samples, and higher dispersion of biological replicates. This effect can be explained by the fact that in poor unreclaimed gravel heaps microbiota has higher sensitivity to micro-scale spatial variation of nutrients introduced by plants, than in primary soils mixed with Agrisol (Ayangbenro and Babalola 2021, Naylor et al. 2020).

Another effect which happens with soil disturbance is the adaptation of microorganisms to the new conditions. It was shown that in treated soils relevance of abundant microorganisms (bacteria and fungi) is reduced and the relevance of low-abundance microorganisms is increased (Bossolani et al. 2021), which can happen since conditions have become less favorable for major microbiota and more favorable for the growth of minor microbiota. We observed this effect in both regions: in comparison to benchmark soils, in disturbed soils major ASV decrease their abundance, while higher quantities of smaller ASV emerge. In Progress microbiomes of disturbed soils still carried the same major ASVs from Firmicutes and Crenarchaeota as in benchmark Agrisol, but their quantities were relatively lower. At the same time, disturbed soils carried many minor ASVs, some of which from cellulose decomposing Chitinophagaceae and Cellulomonas. Presence of these taxa can be linked to increased content of plant residues, which accumulates in quarry soils due to lack of crop harvesting (Kolton et al. 2013).

Soil agrochemical parameters and the microbiome

Content of the most measured agrochemical parameters, including OC, phosphorus, nitrates were low across all sampling sites, which is typical for the local soils (Gorobtsova et al. 2017, Gorobtsova et al. 2021). The acidity of the benchmark and quarry soils corresponds to their genetic features, which is explained by the chemical composition of the mineral waste (Gorobtsova et al. 2016, Gorobtsova et al. 2017, Gorobtsova et al. 2021). In Urvan region main difference between benchmark and quarry soils was reflected in carbon content: benchmark soil retained higher percentages of organic carbon, while primary soils of quarries showed high quantities of total carbon, enhanced by parent rock material. Soil cover in Progress reacts differently to the introduction of parent materials: initial Agrisol already has high quantities of carbon, it rises in the freshly reclaimed quarry

bottom, but is reduced in older quarry bottoms. Organic matter content reduction in disturbed lands with reclamation was reported earlier (Liu et al. 2017).

Benchmark soil in Progress - Agrisol - was the only soil showing prevalence of nitrates over ammonium, which is typical for agricultural soils (Cui and Song 2007). This feature retains in the mining pit neighboring to the field, which showed signs of recent reclamation with the mixture of rock dumps and soil heaps. In the soil of the older quarry the balance of nitrates and ammonium shifts back to ammonium prevalence. It could be linked to soil acidic status, nitrate leaching, or introduction of plant residues since crops were no longer being harvested (Dejoux et al. 2000, Miller and Cramer 2005).

Using several biological replicates with varying agrochemical parameters allowed us to create a reliable model of factors influencing the microbial community. The key factor defining soil disturbance in both regions was OC, which revealed the same ASVs from the two regions reacting to its content - Nitrososphaeracea in benchmark soil, and Nitrososphaeraceae, Azospirillaceae, Cellulomonadaceae, Vicinamibacteraceae, Nocardiodaceae, Chitinophagaceae in quarries. Members of Azospirillaceae were reported to be associated with plants and to be involved in carbon and nitrogen cycles (Sun et al. 2020). Vicinamibacteraceae from Acidobacteria were reported to be flexible in their preferred carbon source (Navarrete et al. 2015). Cellulomonadaceae can degrade not only plant residues, but other carbon sources, like DNA and chitin and (Stackebrandt and Schumann 2014). Nocardiodaceae are considered mostly chemoorganotrophs (Tóth and Borsodi 2014). Thus, with the lack of easy organic carbon in disturbed soil microbiomes are becoming enriched by microbiota, flexible to available energy resources.

Traditionally, microorganisms are divided by their life-history strategy into fast-growing copiotrophs (or r-strategs) and slow-growing oligotrophs (k-strategs) (de Vries and Shade 2013). Based on their growth rates, usually gram-minus soil bacteria, like Proteobacteria, Bacteroidetes, Gemmatimonadetes are treated as copiotrophs, while gram-plus bacteria (Firmicutes, Actinobacteria) as oligotrophs (Fierer et al. 2007, Zhang et al. 2020). However, this division is quite arbitrary and doesn't always follow taxonomical division (Ernebjerg and Kishony 2012, Ho et al. 2017, Song et al. 2017). For example, archaea from *Nitrososphaera* are reported to correlate with nitrate composition in soil (Zhalnina et al. 2014), but in our dataset we found different *Nitrososphaera* ASVs correlating with nitrates, ammonia and organic carbon. Moreover, *Ramlibacter* representatives were described from poor nutrient desert environment (Heulin et al. 2003), and alongside with this fact we found ASV from Gammaproteobacteria - *Ramlibacter* and Ellin6067 - associated with the lack of OC. But there was also other ASVs attributed to *Ramlibacter* and Acidobacteria, associated with the presence of OC. The same trend was detected in Acidobacteria - ASV from Vicinamibacteriaceae, Blastococcaceae were detected in OC-rich soil samples and Vicinamibacteriaceae, *Bryobacter* and RB41 in OC-deprived. So, while prevalence of a certain phyla in the dataset can be linked to some microbiome-forming factors, our analysis showed once again that phyla are formed by phenotypically paradox lower taxons.

Conclusions

Here we described factors influencing microbiome composition of disturbed soils. Disturbance factor acts on the macro-scale level and shapes microbiome of unreclaimed soil almost the same way as the soil type factor. Vegetation brings diversity on the micro-scale level and has higher impact on the unreclaimed soils. Applying of recultivation techniques reduces effect of disturbance and vegetation on the microbiome but does not eliminate it. Soil agrochemical parameters help to explain variation for some groups of microorganisms, regardless macro-scale factors. In Central Caucasus region soil disturbance can be linked to the loss of organic carbon, which reduces representation of major representatives of Firmicutes, and facilitates growth of minor representatives from Acidobacteriota, Actinobacteriota, Bacteroidota and Proteobacteria.

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Author contributions

A.K.K. - sampling, DNA isolation, soil agrochemical data analysis, manuscript preparation, G.V.G. - sampling, microbiome sequencing data analysis, manuscript preparation, R.H.T. – choice of study objects, sampling, A.A.K. - construction and sequencing of the 16S rRNA amplicon libraries, A.G.P. - construction and sequencing of the 16S rRNA amplicon libraries, S.L.H. – manuscript proofreading, E.E.A. – project conceptualisation, manuscript proofreading, E.V.A. – project conceptualisation, manuscript proofreading, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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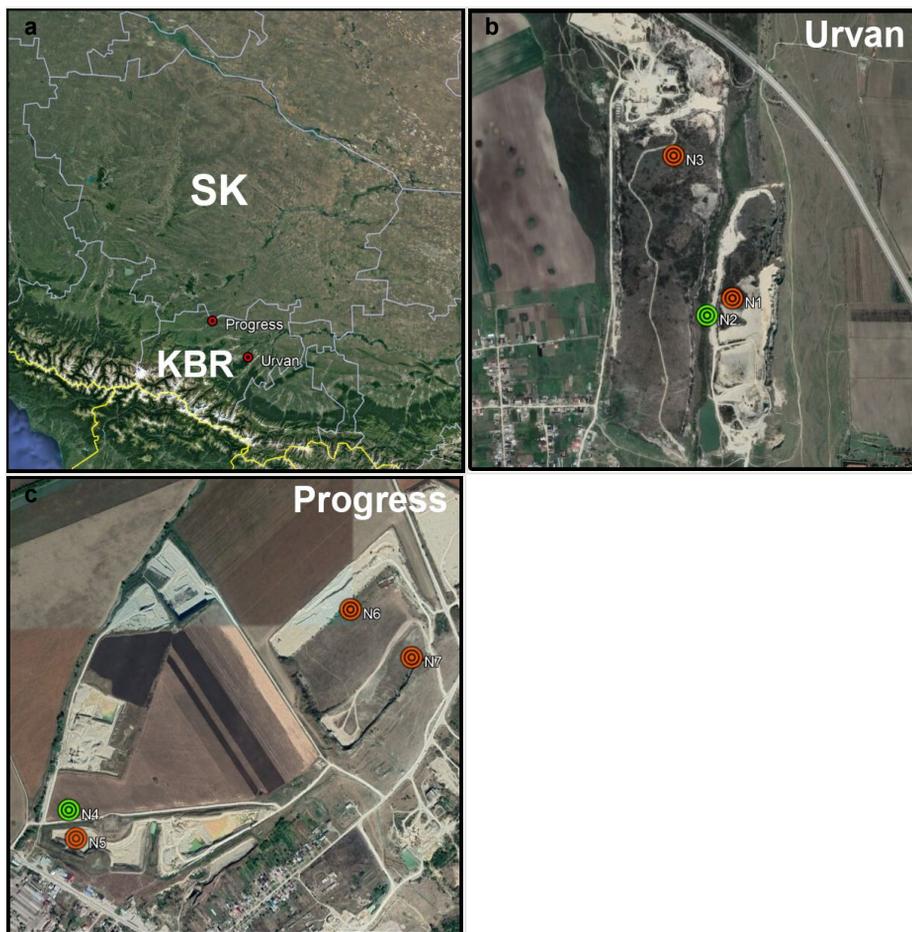


Figure 1.

Sampling sites. Maps based on Google Earth (Google, USA).

a: Relative position of Kabardino-Balkaria Republic (KBR) and Stavropol Krai (SK) regions.

b: Urvan in KBR: N2 benchmark, N1 - quarry 1.1, N3 - quarry 1.2

c: Progress in SK: N4 benchmark 2, N5 - quarry 2.1, N6 and N7 - quarry 2.2

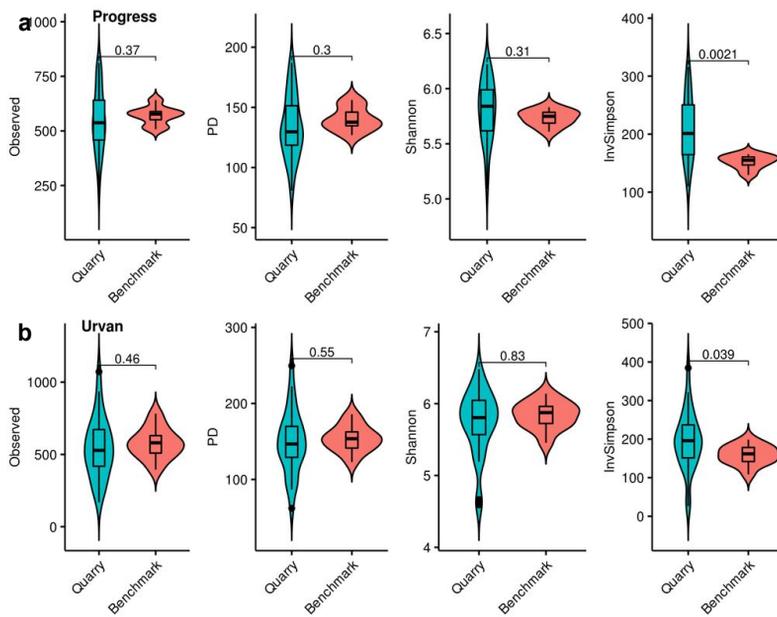


Figure 2.

Alpha diversity in 4 indexes – Observed, Faith (PD), Shannon, Inverted Simpson for quarry/benchmark sites in different regions. Significance of mean differences was calculated by the Mann-Whitney test (Mann and Whitney 1947)

a: Progress region

b: Urvan region

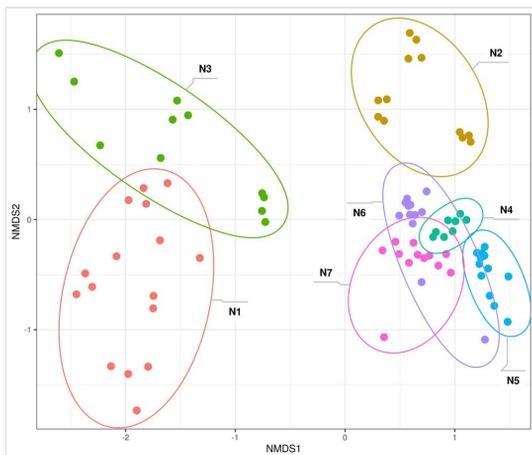


Figure 3.

Beta-diversity of soil sites. Biological and technical replicates of each site are surrounded by ellipses. Urvan: N1, N3 – Quarry, N2 – Benchmark. Progress: N5, N6, N7 – Quarry, N4 – Benchmark.

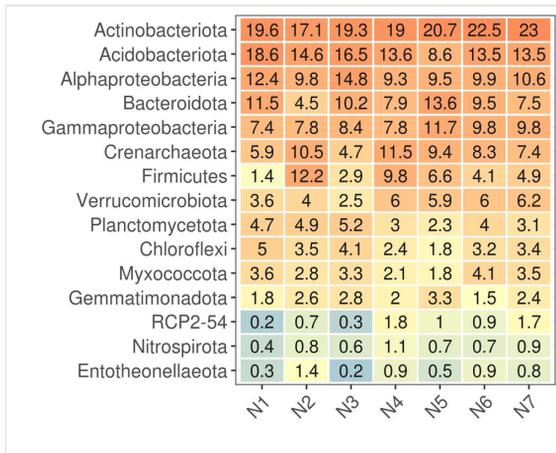


Figure 4.

Heat map of the phyla relative abundance across soil sites. Urvan: N1, N3 – Quarry, N2 – Benchmark. Progress: N5, N6, N7 – Quarry, N4 – Benchmark.

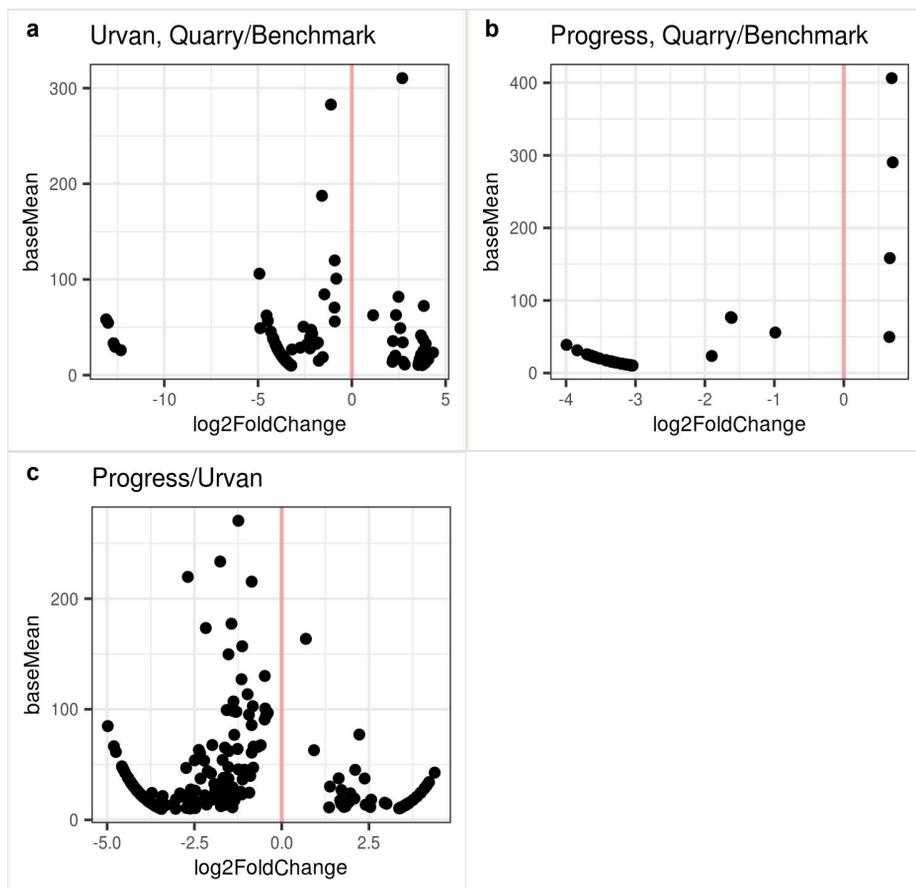


Figure 5.

Plots for DESeq analysis results. Dots represent ASVs, on the Y axis is their base mean, on the X axis \log_2 FoldChange value. The farther the dot is from zero, the stronger the shift between compared groups is, with negative values meaning more of the certain ASV in one group, and positive - in the other.

- a:** \log_2 FoldChange values between quarry and benchmark sites in Urvan
- b:** \log_2 FoldChange values between quarry and benchmark sites in Progress
- c:** \log_2 FoldChange values between sites from different regions

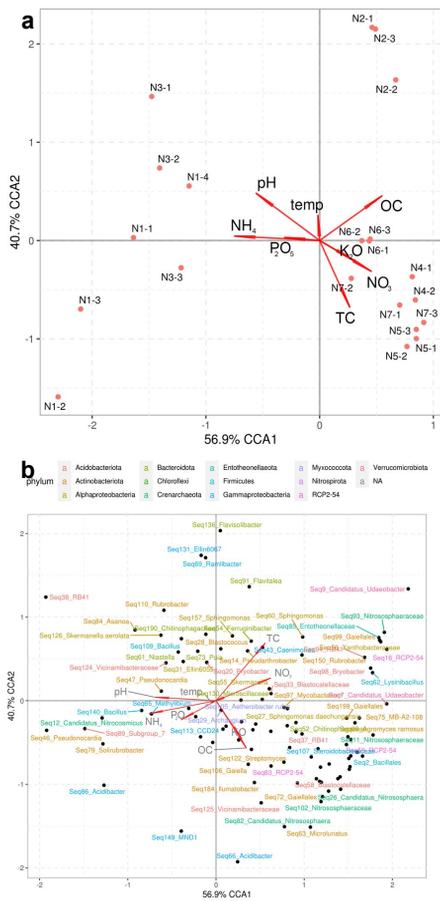


Figure 6.

Plots for CCA analysis, the farther the dot is from the arrow the more it is influenced by the factor. TC – total carbon, OC – organic carbon, temp – temperature scaled to day's mean.

a: Relation of microbiome composition of individual sites to soil agrochemical parameters

b: Relation of the top 100 abundant ASVs to soil agrochemical parameters

Table 1.

Sample description and soil agrochemical parameters with post-hoc Tukey HSD for region and disturbance factors. P-values given in red designate statistically significant influence of a certain factor: region - differences between Urvan and Progress samples, disturbance – between benchmark and primary soils in quarries.

Region	Site	Sample	Temp, C	pH	TC, %	OC, %	P2O5, mg/kg	K2O, mg/kg	NH4, mg/kg	NO3, mg/kg
		Quarry_1.1								
Urvan	N1	N1-1	32	7,7	15	0,29	34,4	259,7	44,96	0,01
		N1-2	25,4	7,4	20	0,56	69,9	360,8	79,93	0,01
		N1-3	35,7	7,4	17	0,34	43	274,2	54,46	0,01
		N1-4	37,4	8	28	0,45	35,2	303	38,5	0,01
		Benchmark_1								
	N2	N2-1	33	7,6	12	1,89	17,7	173,2	15,84	5,2
		N2-2	30	7,5	11	2,71	40,1	389,6	37,34	0,01
		N2-3	34,5	7,6	13	2,69	32,3	288,6	25,59	0,01
		Quarry_1.2								
	N3	N3-1	40	8	18	0,41	16,1	173,2	23,76	0,01
		N3-2	38	7,9	17	0,36	30,9	303	36,55	0,01
		N3-3	28	7,7	18	0,41	44,1	346,3	38,14	0,01
		Benchmark_2								
Progress	N4	N4-1	29	7,1	25	2,56	14	404	13,83	15,6
		N4-2	25	6,9	23	2,21	14,5	389,6	12,37	33,32
		Quarry_2.1								
	N5	N5-1	27	7,5	32	0,78	12,4	331,9	4,75	8,47
		N5-2	25	7,5	21	0,63	15,1	404	6,46	7,8
		N5-3	26	7,6	27	0,65	14	317,5	5,3	13,49
		Quarry_2.2								
	N6	N6-1	28	7,5	15	0,78	41,9	692,6	29,91	0,01
		N6-2	26,5	7,5	16	0,79	42,8	678,2	32,04	0,01
		N6-3	27	7,5	15	0,74	43	793,7	30,16	1,15
		Quarry_2.2								
	N7	N7-1	30	7,3	25	0,87	25,5	606,1	12,67	5,2

	N7-2	31	7,4	24	0,81	80,9	894,7	29,18	0,56
	N7-3	30,5	7,2	27	0,82	21,2	606,1	18,4	0,33
	p-value for Tukey HSD test								
	Region	0,00293	0,00096	0,01465	0,57106	0,40963	0,00081	0,00235	0,00238
	Disturbance	0,93055	0,02319	0,11788	0,00015	0,2228	0,10595	0,20609	0,00245

Table 2.

ASV distribution between soil samples.

	Total			Urvan			Progress		
	Urvan	Common	Progress	Benchmark	Common	Quarry	Benchmark	Common	Quarry
ASV Count	6113	1625	3238	1917	711	5110	534	1064	3265
% of total reads	20,7	65,3	14	17,4	55,9	26,7	2,9	85,7	11,3

Supplementary materials

Suppl. material 1: Supplement figures

Authors: Anastasiia K. Kimeklis, Grigory V. Gladkov, Rustam H. Tembotov, Arina A. Kichko, Alexander G. Pinaev, Sergey L. Hosid, Evgeny E. Andronov, Evgeny V. Abakumov

Data type: images

Brief description: Supporting images of sampling sites and additional analyses

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Suppl. material 2: Supplementary tables

Authors: Anastasiia K. Kimeklis, Grigory V. Gladkov, Rustam H. Tembotov, Arina A. Kichko, Alexander G. Pinaev, Sergey L. Hosid, Evgeny E. Andronov, Evgeny V. Abakumov

Data type: Tables

Brief description: Supplementary tables with sampling site description and statistical analyses

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Suppl. material 3: Supplementary code

Authors: Anastasiia K. Kimeklis, Grigory V. Gladkov, Rustam H. Tembotov, Arina A. Kichko, Alexander G. Pinaev, Sergey L. Hosid, Evgeny E. Andronov, Evgeny V. Abakumov

Data type: code

Brief description: Code used for data analysis

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