

## Research Article

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# Soil microbiome of the postmining areas in polar ecosystems in surroundings of Nadym, Western Siberia, Russia

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**Abstract:** Localization of agriculture with the aim of local food support has become a very urgent topic for Yamal region. The most fertile soils of this region are sandy textured anthropogenically affected soils. Microbiomes from disturbed soils of the Nadym region were studied using analysis of 16S rRNA metagenomic libraries. It was shown that plant cover is a driving force of microbiome composition. Forest soils covered with aeolian transfers from the quarry retains a typical forest microbiome with the following dominant bacterial phyla: Proteobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes and Bacteroidetes. However, it contains significantly less Planctomycetes, which indicates greater aridity of the soil. The microbiomes of the overgrown quarries were strikingly differ from the soil microbiome and resemble those of arctic soils being dominated by Proteobacteria, Chloroflexi, Acidobacteria and Cyanobacteria. Absence of dense vegetation cover and availability of nutrients facilitated the formation of autotrophic microbial mats. The microbiome of the lower horizons of the quarry is characterised by Proteobacteria, Actinobacteria and Firmicutes. Presumably, most of the time these bacteria reside in a dormant state

with short periods of activity due to nutrient uptake from the upper horizons.

**Keywords:** Soils; Polar environments; Microbiome; Agro-landscapes

## 1 Introduction

The soil microbiome plays the most important role in the development of the soil profile and the implementation of key soil-biochemical processes (Will et al. 2010). The soil microbial community is one of the key drivers of implementation of elementary soil processes. This is especially important for the very initial stages of soil regeneration after strong anthropogenic impact (Urbanová et al. 2011; Sprocati et al. 2014). Biogenic-abiogenic interactions are considered as most intensive in the initial stages of soil formation, that is why, initial soil formation could be used as an informative model for parametrization of pedogenesis (Hedenec et al. 2019). Mining areas and related soils represent a very informative subject of primary colonization of various parent materials by numerous groups of microorganisms and this model can be used for development of restoration strategies for post-mining areas (Harantova et al. 2017)

Polar regions usually attracted scientists due to their contrasting and sometimes extreme life conditions. Nevertheless, the severity of climatic conditions are different in various sub zones and sectors of the polar biome. The most popular such polar regions for metagenomic investigations are the moraines of Antarctica (Bottos et al. 2014) and Arctic permafrost soils (Deng et al. 2015; Wilhelm et al. 2011). Terrestrial ecosystems of the Arctic are poorly investigated. Such regions of Russia such as Yamal, Taymyr, and islands of the Northern Ocean are known as regions without essential data on soil microbiome quality (Kirtsideli et al. 2014; 2016, 2017). Generally, these envi-

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ronments represent a unique ecological niche for psychrophilic microorganisms. Together with their microbial population, these soils regularly undergo freeze-thaw cycling, resulting in the stratification of the organic matter and its concentration in the lower soil layers (Jansson and Taş 2014). The most abundant phyla inhabiting these soils are *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes* and *Actinobacteria* (particularly, families *Intrasporangiaceae* and *Rubrobacteriaceae*). Thawing of the permafrost results in changes in the proportion of these phyla, favoring actinobacteria and slowing down the growth of proteobacteria and gemmatimonadetes (Deslippe et al. 2005; Jansson and Taş 2014). The newly discovered phyla, lacking any cultivable representatives, especially phylum AD3, were also described during the analysis of permafrost samples (Frey et al. 2016; Jansson and Tas 2014). A more detailed description of the microbial diversity of permafrost soils can be found in the multiple reviews already published.

Nowadays, fewer studies are devoted to the analysis of soil genesis during the restoration of former mining areas in the Northern regions. At the same time, it is important to understand the structure of microbial communities on the post mine heaps, revealing both the forces driving soil formation and applications in the optimization of the reclamation procedures.

We have found at least two examples of studies targeting soil genesis in the Arctic region. The first is the study of microbiolites and sediments, isolated from an abandoned and flooded open-pit asbestos mine (Yukon, Canada). The analysis of the microbiome composition revealed the dominance of proteobacteria (mainly *Alphaproteobacteria* and *Gammaproteobacteria*), which comprised more than 35% of 16S *rRNA* sequences both in microbiolites and sediments (White et al. 2015). Second is the study of the microbiome's functional and structural composition across a cryo-perturbed polygonal landscape in Alaska. *Proteobacteria* and *Actinobacteria* were the most abundant phyla throughout the soil profiles. *Actinobacteria* comprised up to 68% of the total microbiome and mainly included representatives of the orders *Actinomycetales* and *Solirubrobacterales*. The total amount of these bacteria was correlated with C content and increased substantially in the permafrost layer of soil compared to the active layer. Permafrost layers also had high relative amounts of *Bacteroidetes*, candidate phylum OP9, and archaea from the phylum *Euryarchaeota* (Taş et al. 2018).

Considering the dearth of studies addressing soil genesis of disturbed arctic soils, here we characterize young developing soil of the post-mining landscape of Central Yamal (surroundings of Nadym city) in terms of

the metagenomic composition of soil microbiome. Soils of post mining areas in polar environments are known as possible to be used in agricultural practices after restoration, because they are more drained and more favorable for growing of agricultural production. Areas of sandy textured soils are considered to be expanding with time due to intensive erosion and aeoliation (Sizov and Lobotrosova 2016; Zykina et al. 2017). That is why sands, exposed on the landscape surface are new objects for agricultural practices in the Nadym region. Two ways of involving of these substrata in agriculture are possible: reclamation and further development of local vegetable gardens on the territories of former quarries and soil amendment and creation of arable Agrosols for further plantation of the potatoes. The soil fertility and microbiome composition in the Yamal region is extremely underinvestigated. At the same time the trend for localization of agricultural practices in surroundings of the northernmost cities becomes a very urgent topic of the current research. That is why the aim of this work was to evaluate the taxonomic composition of soil microbiomes on the heaps of former mines in the surroundings of Nadym city, Central part of Yamal, western Siberia, Russia.

The following objectives were formulated in this context: (1) to investigate the soil restoration process in cases of abundant and reclamation practices; (2) to characterize the soil microbial community using the  $\alpha$ - and  $\beta$ -diversity indexes.

## 2 Materials and Methods

### 2.1 The study sites

The study sites are located close to the Nadym city, Yamalo-Nenets autonomous regions, Western Siberia, Russia, on the territory with discontinuous permafrost and covered by natural larch forest. Nadym city and Pangody settlement are located on sand of quaternary age with fine texture and these sands are exposed to intensive wind erosion – aeoliation. The coordinates of the main quarry for sand exploitations are N 65-20-563, E 072-58-135.

### 2.2 Sampling strategy

Six samples of soils associated with quarries were chosen for the metagenomics analysis. These were N1\_R1, N2\_O, N3\_AY, N18\_W, N18\_C1 and N18\_C2. Each sample was analyzed in five replicates. N1-R samples are represented by

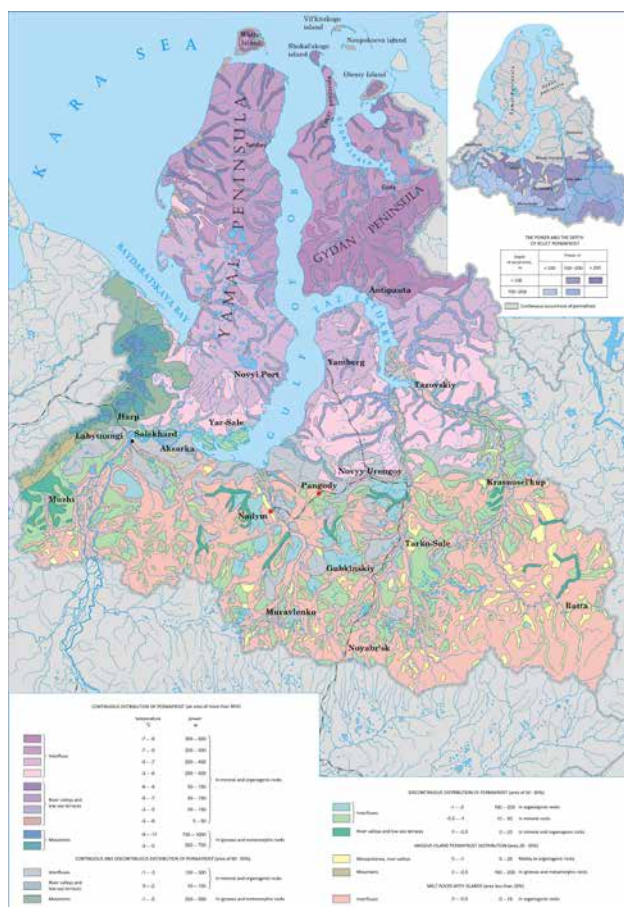


Figure 1: Location of the study sites

former sandy-gravel quarry, where the natural forest was degraded due to the covering of soils and vegetation by aeolian material, which is coming from the quarry. Soil is presented by initial Podzol, covered by aeolian transported sand. The profile structure consists of O horizon of current forest tree stand, sublayered by Aeolian parent material which covers the buried profile of Podzol in a larch forest. Sample N2-O is presented by benchmark soils without aeolian and anthropogenic effect. Sample N3 is presented by sod horizon of initial soil, situated on the bottom of the quarry of sandy-gravel quaternary sediments. Therefore, these three samples are parts of one series of increasing aeolian transportation of sandy-dust materials from the central part of the quarry to the surroundings. Three other samples were collected from the quarry of building materials. This quarry is located on the way from Pangody settlement to Novy Urengoy. Sample N-18 W is weak developed topsoil of Leptosol. This horizon is sub-layered by the horizon of parent material. The sample N18\_C2 is sandy textured parent material from the bottom of the same quarry.

## 2.3 Soil routine analyses

Soils for the routine analyses were grounded and sieved at 2 mm; the mineral samples were dried and sieved at < 2 mm, and the large root debris were picked out manually. Evaluation of carbon, nitrogen, and hydrogen contents were made using a CHN analyzer Leco CHN-628. For the extraction of ammonium nitrogen KCl the EPA method (350.1., 1993) was used. Mobile phosphorus and potassium content was determined using their extraction by 0.5 mol/L HCl (Kuo 1996). The evaluation of the main agrochemical characteristics was performed by the standard procedures GOST 54650-2011 and GOST 26489-85. The first method is based on the extraction of mobile compounds of phosphorus ( $P_2O_5$ ) and potassium ( $K_2O$ ) from the soil with a solution of hydrochloric acid and the subsequent quantitative determination of mobile compounds of phosphorus and potassium. The second method consists of extracting exchangeable ammonium  $NH_4^+$  from the soil with a solution of potassium chloride, and subsequent photometric measurement of the colored solution). Soil basal respiration was determined by a closed chamber experiment under the laboratory conditions.

## 2.4 DNA isolation

Total DNA was isolated from 0.5-0.9 g of the soil samples using the NucleoSpin® Soil Kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the manufacturer's recommendations. For our samples a combination of buffers SL1 and SX was chosen, which is recommended for sandy soils (Lazarevic 2013). Mechanical disintegration of the samples was carried out using a Precellys 24 homogenizer (Bertin Nechnologies, France). The quality of isolation was tested by gel electrophoresis on a 1% agarose gel (Tris-acetate buffer (TAE) 0.5×). DNA concentrations were measured at 260 nm using SPECTROStar Nano (BMG LABTECH, Ortenberg, Germany). The final DNA concentration was, around, 50 ng/μL.

## 2.5 Estimate of microbial quantity with real-time PCR

Real-time PCR was carried out for eubacteria using primers EUB338 5'-ACTCCTACGGGAGGCAGCAG-3' (Lane 1991), EUB518 5'-ATTACCGCGGCTGCTGG-3' (Muyzer et al. 1993), and archaea using primers ARC915f 5'-AGGAATTGGCGGGGAGCAC-3', ARC1059r 5'-GCCATGCACWCCTCT-3' (Yu et al. 2005). The reaction mixture was made using

qPCRMix-HS SYBR kit (Evrogen, Russia) with isolated soil DNA as a template according to the manufacturer's recommendations. Series of 10-fold dilutions of *E. coli* and *H. pylori* 16S rDNA fragments were used as quantity standards. PCR and quantity measurements were carried out using a CFX96 Real-Time PCR Detection System (Bio-Rad, Germany) with the following temperature protocol: 95°C - 3 min; 95°C - 20 s, 50°C - 20 s, 72°C - 20 s (40 cycles).

## 2.6 Constructing and sequencing the 16S rRNA amplicon libraries

Amplicon libraries were prepared using the universal multiplex primers F515 5'-GTGCCAGCMGCCGCGGTAA-3' and R806 5'-GGACTACVSGGTATCTAAT-3' (Bates et al. 2010), targeting the variable region V4 of bacterial and archaeal 16S rRNA genes. Each multiplex primer contained the adapter 4-bp key (TCAG), 10-bp barcode and primer sequences. The reaction mixture was made using Encyclo high-precision polymerase (Evrogen, Russia) with purified DNA preparations, which were used as templates (the temperature profile for PCR was: 95°C - 3 min; 95°C - 30 s, 50°C - 30 s, 72°C - 30 s (30 cycles); 72°C, 30 s). The expected length of the amplification product was 300 bp. Sequencing of the amplicon libraries was carried out using an Illumina MiSeq (Illumina, Inc, USA) in the Centre for Genomic Technologies, Proteomics and Cell Biology (All-Russia Research Institute for Agricultural Microbiology, Russia).

## 2.7 Primary processing of the sequencing data

Trimming, denoising, merging of paired-end reads, deleting of chimera sequences and taxonomic assignment of acquired 16S rRNA gene libraries were performed through "R" and "dada2" packages (Callahan et al. 2016). Phyloge-

netic tree was built using the SEPP package (Janssen et al. 2018) from QIIME2 (Bolyen et al. 2018) on a Silva database (Quast et al. 2013). Analysis of alpha diversity was conducted by Richness, Fisher, Simpson and Shannon indexes using the microbiomeSeq package (Ssekagiri et al. 2017). Beta diversity was assessed by comparison of microbiomes and constructing similarity matrixes between them using weighted and unweighted unifracc (Lozupone et al. 2005) in phyloseq (McMurdie and Holmes 2013), ggplot2 (Wickham 2016), vegan (Oksanen et al. 2017) packages. The significance of differences between microbiomes was estimated by Permanova (Anderson 2017) from the adonis2 test in vegan. Beta diversity was visualized using PCoA in phyloseq. A Mantel test from vegan was performed to test correlation between similarity matrixes and physical parameters of soils. The significance of differences between phylotypes in microbiomes was estimated by model LTR with *fdr* correction by the Benjamin-Hochberg procedure in DESeq2 (Love et al. 2014). Heatmaps were drawn using the ampvis2 tool in R (Albertsen et al. 2015).

## 3 Results

### 3.1 Basic chemical characteristics

Data on routine soil analyzes are given in Table 1. These data indicate that all the soil samples were acidic in reactions from the fine earth. They contain relatively high amounts of total organic carbon and nitrogen. The high content of organic carbon resulted from a low decomposition rate of the topsoil raw materials, accumulated in the forest and initial gross ecosystems. The increased portion of nitrogen could be a result of intensive transformation of the plant remnants in conditions of high aeration of sandy textured soils. The content of available forms of nutrients was not high, but, in general increased portions of nutri-

**Table 1:** Basic agrochemical properties of soils

Sample	pH	TOC, g/kg	N, %	P <sub>2</sub> O <sub>5</sub> , mg/kg	K <sub>2</sub> O mg/kg	N-NH <sub>4</sub> , mg/kg	N-NO <sub>3</sub> , mg/kg	Basal respiration mgCO <sub>2</sub> /100g*day-1
N1_R1	5,45	1.80	0,30	13	148	15,37	0,37	23
N2_O	5,67	2.50	0,60	17	85	0,67	0,37	22
N3_AY	5,34	2.91	0,40	103	63	9,26	0,28	45
N18_W	5,21	7.80	0,60	197	278	27,52	13,70	56
N18_W	5,23	6.70	0,50	87	38	13,56	4,64	45
N18_C2.	5,27	3.41	0,40	48	49	6,64	0,81	32

ents were higher in initial soils, formed on the bottom of quarries, than in benchmark Podzol or in Podzol, partially transformed by the aeolian effect.

### 3.2 Metagenomic study

Six samples of soils associated with quarries were chosen for the metagenomics analysis. These were N1\_R1, N2\_O, N3\_AY, N18\_W, N18\_C1 and N18\_C2. Each sample was analyzed in 5 replicates.

### 3.3 Realtime

Quantification of bacterial operons in purified DNA samples showed, that control forest soil was the richest in bacteria ( $1,37 \cdot 10^{10} \pm 2,63 \cdot 10^9$ ) (Figure 2). Next, in descending order, were N1\_R1 ( $2,9 \cdot 10^9 \pm 2,96 \cdot 10^8$ ), N18\_W ( $1,01 \cdot 10^9 \pm 5,01 \cdot 10^7$ ) and N3\_AY ( $3,93 \cdot 10^8 \pm 3,2 \cdot 10^8$ ). N18\_C1 and N18\_C2 samples had the lowest bacterial operon count, close to negative control response ( $4,77 \cdot 10^6 \pm 8,9 \cdot 10^5$  and  $4,91 \cdot 10^6 \pm 1,47 \cdot 10^6$  respectively). Quantity of archaeal operons was much smaller than that of bacteria but similar in distribution across samples. Maximum quantities were detected for N1\_R1 and N2\_O ( $3,82 \cdot 10^7 \pm 1,11 \cdot 10^7$  and  $3,66 \cdot 10^7 \pm 2,38 \cdot 10^7$ ), medium for N18\_W ( $5,8 \cdot 10^6 \pm 6,96 \cdot 10^5$ ) and N3\_AY ( $2,93 \cdot 10^6 \pm 2,75 \cdot 10^6$ ), and minimum for N18\_C1 ( $3,02 \cdot 10^5 \pm 1,21 \cdot 10^5$ ) and N18\_C2 ( $2,42 \cdot 10^5 \pm 1,47 \cdot 10^5$ ).

Analysis of alpha diversity showed that richness and Fisher indexes for samples from lower horizons (N18\_C1 and N18\_C2) were significantly higher than for upper horizons (N1\_R1/N2\_O and N3\_AY), the same tendency was observed for Shannon index values (Figure 3). Simson index did not differ significantly between sites.

Beta diversity revealed significant differences between different soil samples. Figure 4 presents weighted and unweighted Unifrac on axis 1-2 and 1-3 to show different dimensions. Microbiomes from upper horizons of forest soil (N1\_R1/N2\_O) and microbiomes of lower horizons of second sandy quarry (N18\_C1/N18\_C2) form overlapping pairs. Microbiomes from upper horizons of 2 sandy quarries (N3\_AY/N18\_W pair), however, distinctly separate from each other for both weighted and unweighted Unifrac (overlapping could be seen on axis 1-2 of unweighted unifrac, but it is just one of the dimensions).

Mantel test did not reveal any significant correlation between microbiome composition of different soil samples.

A total of 378904 reads were acquired for 25 libraries of 16S rRNA genes. They were assigned into 4094 phylo-types, of which 4086 (99.8%) were identified on the family level, 2756 (67.4%) on the genus level, 302 (7.4%) on the species level.

Taxonomical composition of microbiomes from forest soils, exposed (N1\_R1) and not exposed (N2\_O) to sand from the quarry appeared to be very similar. The most abundant phyla for both of these soils were Proteobacteria,

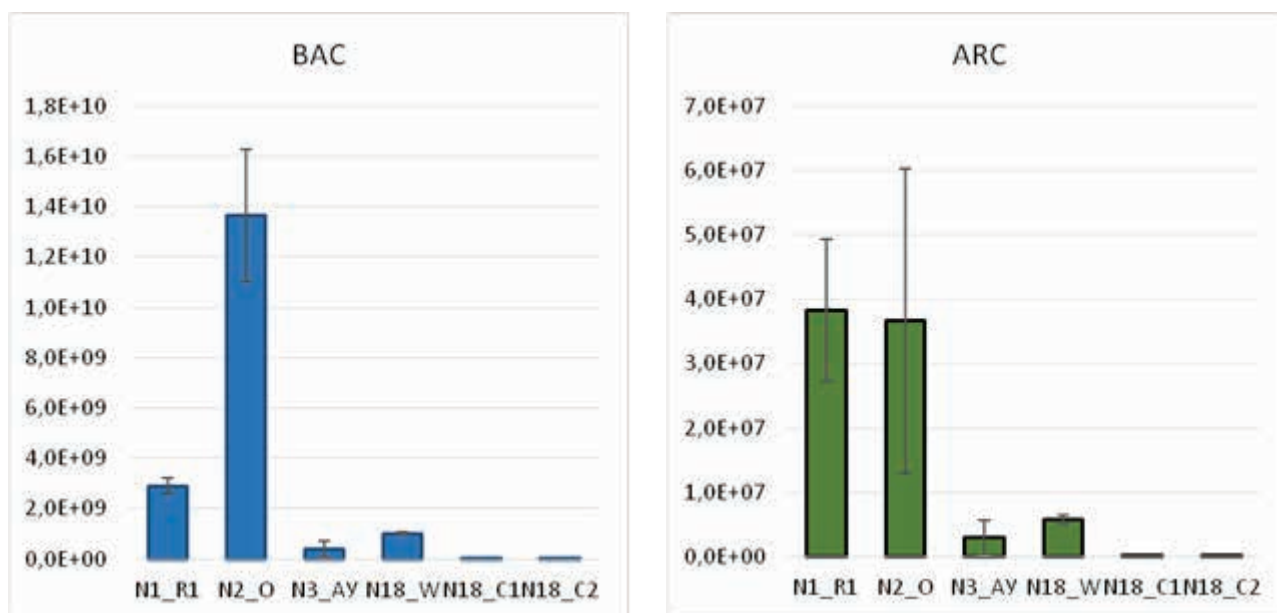


Figure 2: Quantity of bacterial (BAC) and archaeal (ARC) operons in studied soil samples, acquired by Real-time PCR

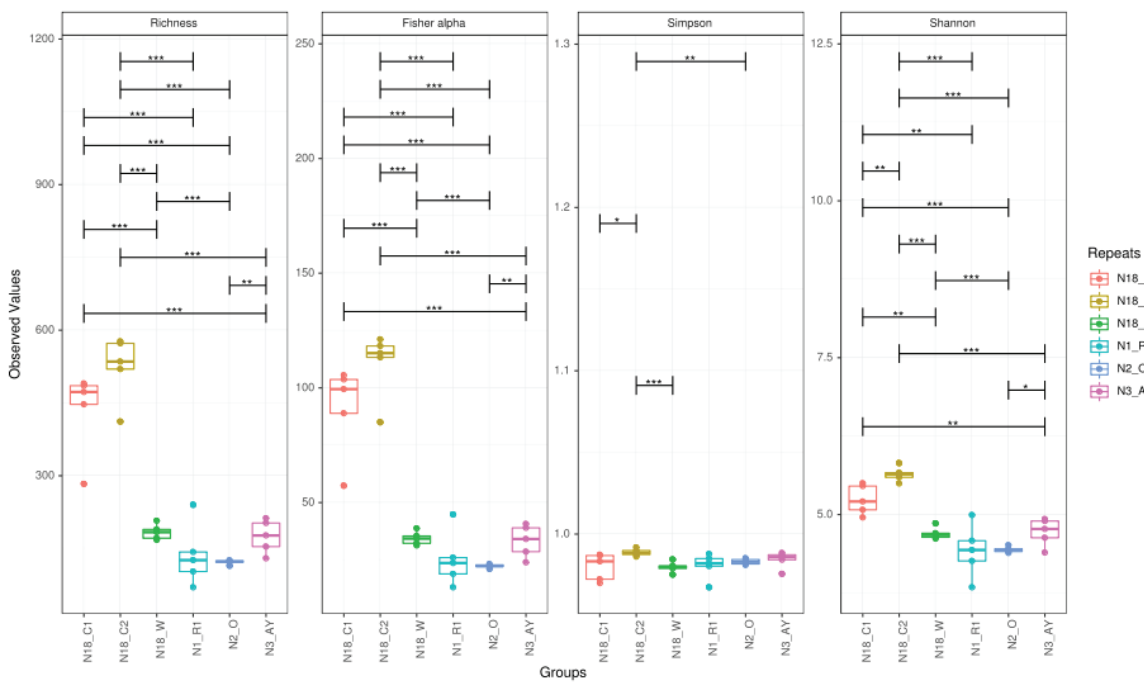


Figure 3: Alpha diversity of samples. Box-plots show value of indexes, lines show significant differences between pairs of samples

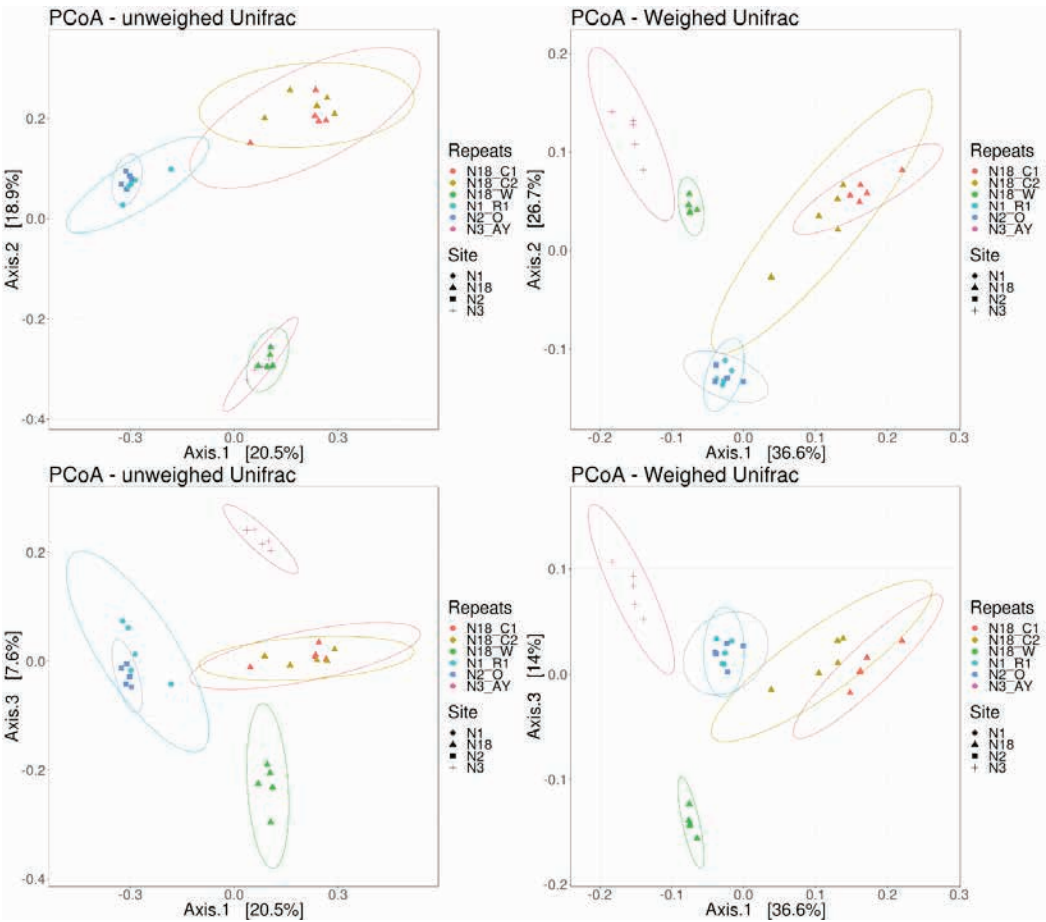


Figure 4: Beta diversity assessed by weighted and unweighted unifracs. PCoA for axis 1-2 and 1-3 are shown to see different dimensions

Proteobacteria -	27.6	36.7
Acidobacteria -	21.9	18.7
Actinobacteria -	14.2	15.2
Verrucomicrobia -	12.6	9.6
Planctomycetes -	8.1	12.1
Bacteroidetes -	12.9	6.7
Chloroflexi -	1.6	0.2
Armatimonadetes -	0.7	0.4
Dependentiae -	0.1	0.2
Chlamydiae -	0.2	0
	$\bar{x}$	$\bar{x}$

**Figure 5:** Top 10 phyla in N1\_R1 and N2\_O samples. Numbers show percentage content of each phylum in a sample

Acidobacteria, Verrucomicrobia, Planctomycetes, Bacteroidetes and Chloroflexi (Figure 5). Significant differences in phylum content were observed for Planctomycetes (more in N2\_O,  $\text{padj}=0.000029$ ), Proteobacteria (more in N2\_O,  $\text{padj}=0.00019$ ) and Bacteroidetes (more in N1\_R1,  $\text{padj}=0.00008$ ).

Taxonomical composition of microbiomes from upper horizons from 2 sandy quarries differed from forest microbiomes and consisted of Proteobacteria, Chloroflexi, Acidobacteria, Cyanobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Planctomycetes, Gemmatimonadetes and Armatimonadetes (Figure 6). Contents of phyla Cyanobacteria, Actinobacteria and Proteobacteria were significantly higher in N18\_W, while contents of Chloroflexi, Verrucomicrobia, and Gemmatimonadetes were significantly higher for N3\_AY.

Microbiomes of upper W and lower C horizons of quarry N18 differed significantly from each other. The most abundant phyla in the lower horizons were Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Acidobacteria, Planctomycetes and Verrucomicrobia (Figure 7). Quantitative composition of C horizons did not differ significantly between each other.

For each set of sample comparisons heatmaps at the level of a phylotype (the lowest taxa level) were built (Figure 8-10). The most prominent differences were observed on the level of families. Control forest soil N2\_O in comparison to aeolian transfers of N1\_R1 had significantly larger amounts of phylotypes from Acetobacteraceae, Isosphaeraceae, Acidobacteriaceae (Subgroup\_1), Burkholderiaceae, Methylococcaceae and Solirubrobacteraceae. N1\_R1 soil horizon was more abundant in Solibacteraceae (Subgroup\_3), Xiphinematobacteraceae, Chitinophagaceae and Sphingobacteriaceae.

Upper horizons of different quarries N3\_AY and N18\_W had more prominent distinctions at the family level. N3\_AY contained significantly more Nostocaceae, Micrococcaceae, Sphingomonadaceae, Leptolyngbyaceae, Acetobacteraceae, Chitinophagaceae and AKIW781. N18\_W contained more Ktedonobacteraceae, Chthoniobacteraceae, Gemmatimonadaceae, WD2101\_soil\_group, Xiphinematobacteraceae and Pedosphaeraceae.

Microbiomes of upper W and lower C horizons of quarry N18 differed from each other even more significantly at the level of families than the level of phyla. Composition of lower horizons microbiomes, though, were quite similar.



Figure 6: Top 10 phyla in N18\_W and N3\_AY samples. Numbers show percentage content of each phylum in a sample

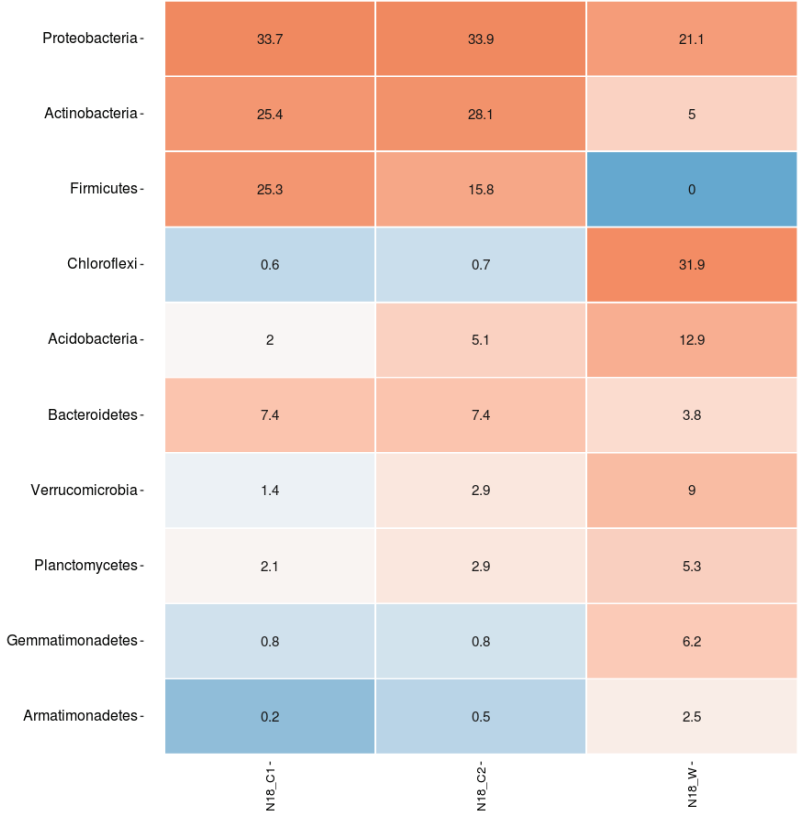


Figure 7: Top 10 phyla in 3 horizons of N18 soil sample. Numbers show percentage content of each phylum in a sample

Solibacteraceae_(Subgroup_3); Bryobacter-	9.4	2.5
Acidobacteriaceae_(Subgroup_1); Granulicella-	4.9	6.9
Xanthobacteraceae; f__Xanthobacteraceae_Seq3-	5.6	6
Acidothermaceae; Acidothermus-	5.3	5.9
Mycobacteriaceae; Mycobacterium-	5	5.3
Burkholderiaceae; Burkholderia-Caballeronia-Paraburkholderia-	3.9	6.3
Isosphaeraceae; Aquisphaera-	3.6	5.6
Xiphinematobacteraceae; Candidatus_Xiphinematobacter-	6.5	2.6
Acidobacteriaceae_(Subgroup_1); Acidipila-	3.4	3.6
Sphingobacteriaceae; Mucilaginibacter-	5	1.6
Chitinophagaceae; f__Chitinophagaceae_Seq34-	3.3	2.9
Beijerinckiaceae; Roseiarcus-	2.1	2.3
Isosphaeraceae; Singulisphaera-	0.6	3.4
Solibacteraceae_(Subgroup_3); Candidatus_Solibacter-	2.3	1.4
Acetobacteraceae; Acidocella-	0.2	3.6
Chthoniobacteraceae; Chthoniobacter-	1.1	2.4
Acetobacteraceae; f__Acetobacteraceae_Seq52-	1.2	2
Acidobacteriaceae_(Subgroup_1); Edaphobacter-	1.2	2
Solirubrobacteraceae; Conexibacter-	1.2	2
Acetobacteraceae; f__Acetobacteraceae_Seq67-	1.2	2
Acetobacteraceae; Acidicoccus-	1.9	1
Chitinophagaceae; f__Chitinophagaceae_Seq92-	2.3	0.4
Unknown_Family; Acidibacter-	1.6	1
Chthoniobacteraceae; Candidatus_Udaeobacter-	2.3	0.1
Burkholderiaceae; Pandoraea-	0.3	1.9
Microbacteriaceae; Galbitalea-	1.3	0.6
Methylacidiphilaceae; f__Methylacidiphilaceae_Seq171-	1.1	0.8
Polyangiaceae; Pajaroellobacter-	0.5	0.9
Xanthobacteraceae; f__Xanthobacteraceae_Seq194-	1.4	0
Xanthobacteraceae; f__Xanthobacteraceae_Seq219-	0.1	1.1
	N1	N2

**Figure 8:** Heatmap of the 30 most abundant phylotypes for samples N1\_R1 and N2\_O

## 4 Discussion

The dataset consisted of 6 soil samples. It included a sample of forest soil near quarry N2\_O, which could be considered as a control for sample N1\_R1, which came from the same forest soil, but was covered with aeolian transfers from the quarry, and sample N3\_AY, which was taken from the overgrown quarry. Another set of samples came from a different sandy quarry and represented 3 horizons of the overgrown quarry bottom.

The microbiome of the N2\_O sample showed typical composition of a forest soil. Analysis of N1\_R1 sample

showed that aeolian transfers from the quarry acquired microbiome composition, very similar to the forest soil. Differences in Planctomycetes content in these samples could point out that unexposed forest soil is more waterlogged than forest soil, exposed to sand (Dedysh and Ivanova 2019). The N2\_O soil microbiome was more abundant in *Mycobacterium* and *Pandoraea alpina*, which inhabit rhizosphere. N1\_R1 microbiomes consisted of more copiotrophs (*Bryobacter*, *Candidatus\_Xiphinematobacter*) and bacteria tolerant to heavy metals (*Chitinophagaceae*).

The microbiome of the overgrown quarry (N3\_AY), which was the source for aeolian transfers, is distinctly

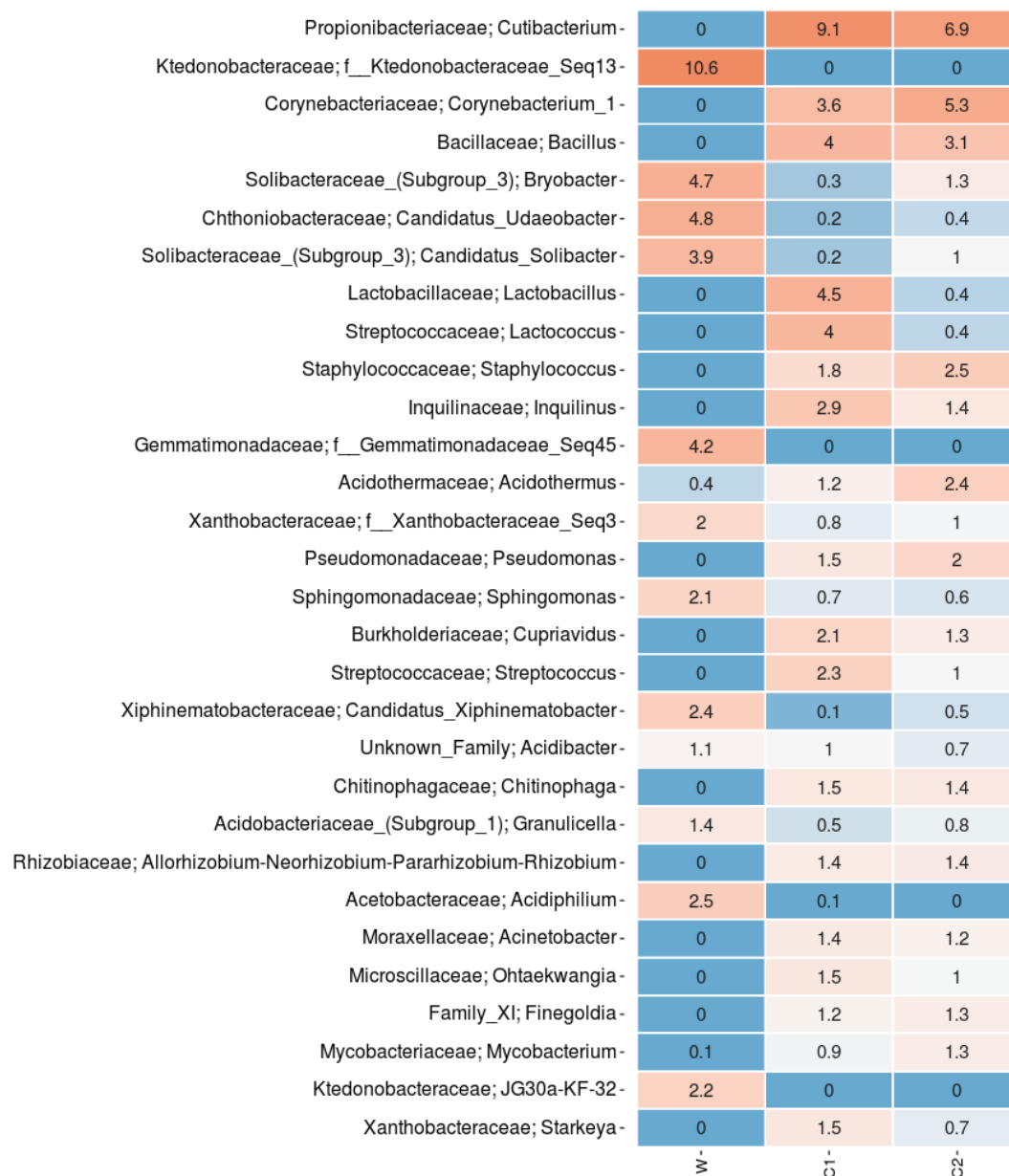
Nostocaceae; Mastigocladopsis_PCC-10914-	0.5	10.6
Acetobacteraceae; Acidiphilium-	2.5	8.1
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq13-	10.6	0
Solibacteraceae_(Subgroup_3); Candidatus_Solibacter-	3.9	5.5
Solibacteraceae_(Subgroup_3); Bryobacter-	4.7	2.5
Sphingomonadaceae; Sphingomonas-	2.1	3.5
Chthoniobacteraceae; Candidatus_Udaeobacter-	4.8	0.6
Chthonomonadaceae; Chthonomonas-	1.7	2.8
Gemmatimonadaceae; f_Gemmatimonadaceae_Seq45-	4.2	0.3
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq54-	0	4.2
Acidobacteriaceae_(Subgroup_1); Granulicella-	1.4	2.4
Micrococcaceae; Pseudarthrobacter-	0.3	2.7
Ktedonobacteraceae; JG30a-KF-32-	2.2	0.6
Gemmatimonadaceae; Gemmatimonas-	1.3	1.4
Nostocaceae; Stigonema_SAG_48.90-	0	2.7
Chitinophagaceae; Flavisolibacter-	0.5	2
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq90-	2	0.5
Xanthobacteraceae; f_Xanthobacteraceae_Seq3-	2	0.5
Xiphinematobacteraceae; Candidatus_Xiphinematobacter-	2.4	0
Acidobacteriaceae_(Subgroup_1); Acidipila-	0.9	1.5
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq112-	2.1	0
Ktedonobacteraceae; G12-WMSP1-	1.9	0.1
Sphingobacteriaceae; Mucilaginibacter-	1.3	0.8
Archangiaceae; Anaeromyxobacter-	0.6	1.4
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq125-	1.9	0
Polyangiaceae; Pajaroellobacter-	0.6	1.2
Chitinophagaceae; f_Chitinophagaceae_Seq140-	0.5	1.3
Ktedonobacteraceae; f_Ktedonobacteraceae_Seq154-	0.9	0.8
Catenulisporaceae; Catenulispora-	1.1	0.4
Unknown_Family; Acidibacter-	1.1	0.4
	$\frac{1}{N}$	$\frac{2}{N}$

**Figure 9:** Heatmap of the 30 most abundant phylotypes for samples N18\_W and N3\_AY

different from the forest soil (N2\_O). Microbiomes from two different sandy quarries (N3\_AY and N18\_W) turned out to be more similar, than microbiomes from close, but different environments (N3\_AY and N2\_O). Composition of sandy quarry microbiomes was close to arctic soils (Gilichinsky et al. 2007). Prevalence of Chloroflexi and Cyanobacteria allow us to suggest that the overgrown quarry is covered in microbial mats, which consist mostly of autotrophs. These mats are primary inhabitants of soils. Both N18\_W and N3\_AY sites are rich in nutrients, and supposedly without plant cover, to reduce levels of available nitrogen, the microbiome was occupied by autotrophs. Usually such mats consist of different layers, and

Cyanobacteria, as aerobes, occupy its upper layers, while Chloroflexi, as anaerobes, occupy its bottom layers. In our samples one quarry had mostly only Chloroflexi (Ktedonobacteraceae) and the other mostly only Cyanobacteria (Stigonema\_SAG\_48.90, Mastigocladopsis\_PCC-10914, Mastigocladopsis\_PCC-10914, Nostoc\_PCC-73102). One of the reasons could be that the extent of sampling was insufficient to detect all components of a microbial mat in each sample. N18\_W is richer in nitrogen than N3\_W, so another reason could be that Chloroflexi, being mixotrophs, pushed out Cyanobacteria from their niche.

Soil samples from C horizons of N18 quarry had miniscule amounts of DNA. It was hard to get PCR products of



**Figure 10:** Heatmap of the 30 most abundant phylotypes for 3 horizons of N18 soil sample

16S rRNA gene for these samples and the resulting libraries were very small. However, alpha diversity of these samples was much higher than of all other samples. It is possible, that microbiome of deep layers are dependent on the rare periodical washings of nutrients from upper layer, which leads to formation of an oligotrophic persists community from Firmicutes phylum, which can grow in the periods of nutrients availability and wait in dormancy during starving conditions.

Physical parameters of all soil plots differed from each other, but Mantel test did not show significant correlation of them with microbiome composition. Perhaps, this result comes not from the actual absence of correla-

tion, but from high heterogeneity of microbiomes datasets (Anderson and Walsh 2013).

## 5 Conclusions

Soils, associated with former quarries in the polar terrestrial environments of the Nadym region are considered as potential agricultural lands. The benchmark Podzols of this region are very poor in terms of key nutrients content. In contrast, soils formed of the bottoms of the quarries of the quaternary sediment are known as more fertile

due to the absence of nutrients leaching. That is why we have investigated the soil microbial community in soils of quarries and natural environments. It was shown that plant cover is a driving force of microbiome composition. Natural forest soil covered with aeolian dusts from the sandy-gravel quarry retains a typical forest microbiome with the following dominant bacterial phyla: Proteobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes and Bacteroidetes. However, it contains significantly less Planctomycetes, which indicates greater aridity of the soil. Microbiome of the abandoned overgrown quarries strikingly differ from soil microbiome and resemble those of arctic soils with dominant Proteobacteria, Chloroflexi, Acidobacteria and Cyanobacteria. Absence of dense vegetation cover and availability of nutrients facilitated the formation of autotrophic microbial mats. The microbiome of lower horizons of the quarry soil is characterized of Proteobacteria, Actinobacteria and Firmicutes. Supposedly, most of the time these bacteria reside in a dormant state with short periods of activity due to nutrient uptake from the upper horizons. Therefore, huge redistribution of the Aeolian dust from the quarries could essentially change the natural soil quality both in terms of nutrient state and microbial communities. In this context, the use of redevelopment of agricultural practices which had appeared recently on the landscapes, formed on sandy textured parent materials, should take into account data on microbial communities of anthropogenic soils.

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## Rereferences

- [1] Albertsen M., Karst S.M., Ziegler A.S., Kirkegaard R.H., Nielsen P.H., Back to Basics – The Influence of DNA Extraction and Primer Choice on Phylogenetic Analysis of Activated Sludge Communities, *PLOS ONE*, 2015, 10(7), e0132783, doi:10.1371/journal.pone.0132783
- [2] Anderson, M. J. and Walsh, D. C., PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, 2013, 83, 557-574, doi:10.1890/12-2010.1
- [3] Anderson, M.J., Permutational Multivariate Analysis of Variance (PERMANOVA), in *Wiley StatsRef: Statistics Reference Online* (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorisch, F. Ruggeri and J. L. Teugels), 2017, doi:10.1002/9781118445112.stat07841
- [4] Bates S.T., Berg-Lyons D., Caporaso J.G., Walters W.A., Knight R., Fierer N., Examining the global distribution of dominant archaeal populations in soil, *ISME J*, 2010, 5, 908–917, doi:10.1038/ismej.2010.171.
- [5] Bolyen E., Rideout J.R., Dillon M.R., Bokulich N.A., Abnet C., Al-Ghalith G.A., Alexander H., Alm E.J., Arumugam M., Asnicar F., Bai Y., Bisanz J.E., Bittinger K., Brejnrod A., Brislawn C.J., Brown C.T., Callahan B.J., Caraballo-Rodríguez A.M., Chase J., Cope E., Da Silva R., Dorrestein P.C., Douglas G.M., Durall D.M., Duvallet C., Edwardson C.F., Ernst M., Estaki M., Fouquier J., Gauglitz J.M., Gibson D.L., Gonzalez A., Gorlick K., Guo J., Hillmann B., Holmes S., Holste H., Huttenhower C., Huttley G., Janssen S., Jarmusch A.K., Jiang L., Kaehler B., Kang K.B., Keefe C.R., Keim P., Kelley S.T., Knights D., Koester I., Kosciulek T., Kreps J., Langille M.G., Lee J., Ley R., Liu Y., Loftfield E., Lozupone C., Maher M., Marotz C., Martin B.D., McDonald D., McIver L.J., Melnik A.V., Metcalf J.L., Morgan S.C., Morton J., Naimey A.T., Navas-Molina J.A., Nothias L.F., Orphanian S.B., Pearson T., Peoples S.L., Petras D., Preuss M.L., Priesse E., Rasmussen L.B., Rivers A., Robeson II M.S., Rosenthal P., Segata N., Shaffer M., Shiffer A., Sinha R., Song S.J., Spear J.R., Swafford A.D., Thompson L.R., Torres P.J., Trinh P., Tripathi A., Turnbaugh P.J., Ul-Hasan S., van der Hooft J.J., Vargas F., Vázquez-Baeza Y., Vogtmann E., von Hippel M., Walters W., Wan Y., Wang M., Warren J., Weber K.C., Williamson C.H., Willis A.D., Xu Z.Z., Zaneveld J.R., Zhang Y., Zhu Q., Knight R., Caporaso J.G., QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science, *PeerJ Preprints*, 2018, 6, e27295v2, doi:10.7287/peerj.preprints.27295v2
- [6] Bottos E.M., Scarrow J.W., Archer S.D.J., McDonald I.R., Cary S.C., Bacterial Community Structures of Antarctic Soils, in Cowan, D.A. (ed) *Antarctic Terrestrial Microbiology: Physical and Biological Properties of Antarctic Soils*, 2014, Springer-Verlag Berlin, Heidelberg, 9–33, doi:10.1007/978-3-642-45213-0.
- [7] Callahan B.J., McMurdie P.J., Rosen M.J., Han A.W., Johnson A.J.A., Holmes S.P., High-resolution sample inference from Illumina amplicon data, *Nature Methods*, 2016, 13, 581-583
- [8] Dedysh S.N., Ivanova A.A., Planctomycetes in boreal and subarctic wetlands: Diversity patterns and potential ecological functions, *FEMS Microbiol Ecol.*, 2019, 95(2), doi:10.1093/femsec/fiy227
- [9] Deng J., Gu Y., Zhang J., Xue K., Qin Y., Yuan M., Yin H., He Z., Wu L., Schuur E.A., Tiedje J.M., Zhou J., Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska. *Mol Ecol.*, 2015, 24, 222-234, doi:10.1111/mec.13015
- [10] Deslippe J.R., Egger K.N., Henry G.H.R. Impacts of warming and fertilization on nitrogen-fixing microbial communities in the Canadian High Arctic, *FEMS Microbiology Ecology*, 2005, 53, 41–50, doi:10.1016/j.femsec.2004.12.002.
- [11] EPA method 350.1, Determination of Ammonia Nitrogen by automated colorimetry. Revision 2.0, 1993, 14.
- [12] Gilichinsky D., Wilson G., Friedmann E., McKay C., Sletten R., Rivkina E. Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology, *Astrobiology* 2007, 7, 275–311

- [13] GOST 26489-85. Soils. Determination of exchangeable ammonium by CINA method, 1985, 5
- [14] GOST 54650-2011. Soils. Determination of mobile phosphorus and potassium compounds by Kirsanov method modified by CINA, 2011, 8
- [15] Harantová, L., Mudrák, O., Kohout, P., Elhottová, D., Frouz, J., Baldrian, P., Development of microbial community during primary succession in areas degraded by mining activities, *Land Degradation and Development*, 2017, 28(8), 2574-2584
- [16] Heděný, P., Jílková, V., Lin, Q., Cajthaml, T., Filipová, A., Baldrian, P., Větrovský, T., Křišťálek, V., Chroňáková, A., Setälä, H., Tsiafouli, M.A., Mortimer, S.R., Kukla, J., Frouz, J., Microbial communities in local and transplanted soils along a latitudinal gradient *Catena*, 2009, 73, 456-464
- [17] Janssen S., McDonald D., Gonzalez A., Navas-Molina Jose A., Jiang L., Xu Z.Z., Winker K., Kado D.M., Orwoll E., Manary M., Mirarab S., Knight R., Phylogenetic Placement of Exact Amplicon Sequences Improves Associations with Clinical Information, *Systems*, 2018, doi:10.1128/mSystems.00021-18
- [18] Jansson J.K., Tas N., The microbial ecology of permafrost, *Nature Reviews Microbiology*, 2014, 12, 414-425, doi:10.1038/nrmicro3262
- [19] Kirtsideli I.Y., Vlasov D.Yu., Barantsevitch E.P., Krylenkov V.A., Sokolov V.T., Microfungi from soil of polar Island Izvestia tsik (Kara sea), *Mikologiya i Fitopatologiya*, 2014, 48, 365-371
- [20] Kirtsideli I.Yu., Abakumov E.V., Barantsevitch E.P., Krylenkov V.A., Sokolov V.T., Microbial communities in regions of arctic settlements, *Gigiena i Sanitariya*, 2016, 95, 923-929
- [21] Kirtsideli I.Yu., Vlasov D.Yu., Abakumov E.V., Barantsevitch E.P., Novozhilov Y.K., Krylenkov V.A., Sokolov V.T. Airborne fungi in arctic settlement Tiksi (Russian Arctic, coast of the Laptev Sea), *Czech Polar Reports*, 2017, 7, 300-310, doi:10.5817/CPR2017-2-29
- [22] Frey B., Rime T., Stierli B., Hajdas I., Widmer F., Hartmann M., Microbial diversity in European alpine permafrost and active layers, *FEMS Microbiology Ecology*, 2016, 92, 1-17, doi:10.1093/femsec/fiw018
- [23] Kuo S., Phosphorus, in: Bartels JM, Bigham JM. *Methods of Soil Analysis*, 3. Chemical Methods. Madison, Soil Science Society of America, 1996, 869-919
- [24] Lazarevic V., Gaia N., Girard M., Francois P., Schrenzel J., Comparison of DNA Extraction Methods in Analysis of Salivary Bacterial Communities, *PLoS One*, 2013, 8(7), 67699, doi:10.1371/journal.pone.0067699
- [25] Lane D.J. 16S/23S rRNA Sequencing, in: *Nucleic Acid Techniques in Bacterial Systematic* /Stackebrandt E., Goodfellow, M. (eds.). John Wiley and Sons, New York, 1991
- [26] Lozupone C., Knight R., UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, 2005, 71, 8228-8235, doi:10.1128/AEM.71.12.8228-8235
- [27] Love M.I., Huber W., Anders S., Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome Biol.*, 2014, 15(12), 550, doi:10.1186/s13059-014-0550-8
- [28] Muyzer G., de Waal E.C., Uitterlinden A.G., Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, *Applied and Environmental Microbiology*, 1993, 59(3), 695-700
- [29] Oksanen J., Blanchet F.G., Friendly M., Kindt R., Legendre P., McGlinn D., *vegan: Community Ecology Package*. R Package Version 2.3-3, 2017, <http://CRAN.R-project.org/package=vegan>
- [30] Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., Glöckner F.O., The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Research*, 2013, 41(1), 590-596, doi:10.1093/nar/gks1219
- [31] McMurdie and Holmes, phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data, *PLoS ONE*, 2013, 8(4), e61217
- [32] Sizov O.S., Lobotrosova S.A. Features of revegetation of drift sand sites in the Northern Taiga subzone of Western Siberia, *Earth's Cryosphere*, 2016, 20(3), 3-13
- [33] Sprocati A.R., Alisi C., Tasso F., Bioprospecting at former mining sites across Europe: microbial and functional diversity in soils, *Environ Sci Pollut Res*, 2014, 21, 6824, doi:10.1007/s11356-013-1907-3
- [34] Urbanova M., Kopecky J., Valaskova V., Sagova-Marechikova M., Elhottova D., Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining, *FEMS Microbiology Ecology*, 2011, 78, 59-69, 10.1111/j.1574-6941.2011.01164.x.
- [35] Taş, N., Prestat, E., Wang, S., Landscape topography structures the soil microbiome in arctic polygonal tundra, *Nat Commun*, 2018, 9, 777, doi:10.1038/s41467-018-03089-z
- [36] White R.A. 3rd, Power I.M., Dipple G.M., Southam G., Suttle C.A., Metagenomic analysis reveals that modern microbialites and polar microbial mats have similar taxonomic and functional potential, *Front Microbiol.*, 2015, 6, 966, doi:10.3389/fmicb.2015.00966
- [37] Wickham. H., *ggplot2: Elegant Graphics for Data Analysis*, Springer-Verlag, New York, 2016.
- [38] Wilhelm R.C., Niederberger T.D., Greer C., Whyte L.G., Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic, *Can J Microbiol*, 2011, 57, 303-315
- [39] Will C., Thurmer A., Wollherr A., Nacke H., Herold N., Schrupf M., Gutknecht J., Wubet T., Buscot F., Daniell R., Horizon-specific bacterial community composition of german grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes, *Applied and Environmental Microbiology*, 2010, 76, 6751-6759, doi:10.1128/AEM.01063-10
- [40] Yu Y., Lee C., Hwang, S., Analysis of community structures in anaerobic processes using quantitative real-time PCR method, *Water science and technology: a journal of the International Association on Water Pollution Research*, 2005, 52, 85-91, doi:10.2166/wst.2005.0502
- [41] Zykina, V.S., Zykin, V.S., Volvach, A.O., Ovchinnikov, I.Yu., Sizov, O.S., Soromotin, A.V., Upper quaternary deposits of the nadym ob area: Stratigraphy, cryogenic formations, and deposition environments, *Earth's Cryosphere*, 2017, 21(6), 14-25