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Soil microbiomes of reclaimed and abandoned mines of the Yamal region

Elizaveta PERSHINA [1,2], Ekaterina IVANOVA [2,3,4], Anastasia KIMEKLIS[2], Alexey ZVEREV., Arina KICHKO[2], Tatiana AKSENOVA[2], Evgeny ANDRONOV [1,2] and Evgeny ABAKUMOV [1,*]

[1] *Saint-Petersburg State University, Saint-Petersburg, Russia*

[2] *All-Russia Research Institute for Agricultural Microbiology, Saint-Petersburg, Russia*

[3] *Dokuchaev Soil Institute, Moscow, Russia*

[4] *Agrophysical Research Institute, Saint-Petersburg, Russia*

[*] *corresponding author <e_abakumov@mail.ru>*

Abstract: Here we investigate the microbiomes of the soil samples from the Yamal Peninsula (the surroundings of Salekhard city, Russian Federation) using a high-throughput sequencing approach. The main goal was to investigate the impact of mining on soils within the following regeneration, both during the reclamation practice and natural self-growth. Several quarries were studied, engaged in sand, clay and chromatic ores mining. The taxonomic analysis of the soil microbiomes revealed 50 bacterial and archaeal phyla; among the dominant phyla were: Proteobacteria, Actinobacteria, Acidobacteria, Chroloflexi, Gemmatimonadetes, Verrucomicrobia, Planctomycetes, Bacteroidetes, AD3, and Nitrospirae. Compared to the typical tundra soil, which was chosen as a control, the disturbed soils had increased biodiversity and total counts for soil bacteria, archaea, and fungi, especially in the cryosolic horizon. The different mining strategies caused significantly different transformations of soil microbiomes, which was less pronounced for self-growth compared to reclaimed quarries. This isolation of the reclaimed quarry was mainly associated with the increase of the amount of acidobacteria (fam. Koribacteraceae and Acidobacteriaceae and order Ellin6513), some proteobacterial taxa (fam. Syntrophobacteraceae), and Chloroflexi (fam. Thermogemmatisporaceae). The study also revealed bacteria, which tend to be specific for marine tundra environments: gemmatimonadetes from the order N1423WL and Chloroflexi bacteria from the order Gitt-GS-136.

Keywords: Arctic, Yamal Peninsula, microbiome, soil, high-throughput sequencing, 16S rRNA, qPCR, mining, reclamation.

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). Key soil forming processes are connected with activity of soil microbiome directly or indirectly. This is especially important for the very initial stages of soil regeneration after strong anthropogenic impact (Urbanová *et al.* 2011; Sprocati *et al.* 2014).

Polar regions usually attracted scientists due to their contrasting and sometimes extreme life conditions. The most popular such polar regions are the moraines of Antarctica (Bottos *et al.* 2014) and Arctic permafrost soils (Deng *et al.* 2015). The latter can be treated as a similar environment to the poorly investigated regions in Russia: the Yamal, Taymyr, and islands of the Northern Ocean. Generally, these environments 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 leads to 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 (Jansson and Taş 2014; Frey *et al.* 2016). More detailed description of the microbial diversity of permafrost soils can be found in the multiple published reviews.

Contrary, much 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 quite important to understand the structure of microbial communities on post mine heaps, both understanding the forces driving soil formation and applications in the optimization of the reclamation procedures. Soil restoration in an initial stage is drived by few groups of microorganisms, which can be considered as microbial drivers of soil formation. This is especially important for initial stages of abiogenic-biogenic interactions in soil and soil like bodies, formed on the surfaces of heaps and mines. At the same time, data on soil microbial diversity, based both on classical methods of laboratory cultivation and new approaches of metagenomics studies, are considered as not enough for polar anthropogenically disturbed environments. New generation sequencing allows investigating completely new levels of biodiversity anthropogenic environments than classical methods of laboratory cultivation of microorganisms (Pershina *et al*. 2019). Previously, this method was recognized as informative and productive tool for investigation of soil microbiome on the mines in boreal

environments (Dmitrakova *et al.* 2018 a, b). We manage to find 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 microbiome functional and structural composition across a cryoperturbed 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). Thus, investigation of taxonomy structure of soil microbiome become the first required stage for understanding possible drivers of soil processes and to finding possible fingerprints for environmental changes on soil quality and biological properties. New generation sequencing techniques allow obtaining principally new quality of data, regarding soil microbial diversity. 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 in terms of the metagenomic composition of soil microbiome. The following scientific objectives were formulated to achieve the aim: (1) to investigate the soil restoration process in cases of abundant and reclamation practices; (2) to characterize soil microbial community using the alfa- and beta-diversity indexes.

Materials and methods

The study sites located in central part of Yamal region, in southern part of Yamal Peninsula close to the city of Salekhard – capital of Yamal region (Table 1, Fig. 1). This location causes the abundance of number of formed and currently active mined and quarries, characterized by exposure of minerals of various texture and composition on the surface of mature tundra landscape (Fig. 2). Hummock tundra and forest-tundra communities dominate the vegetation cover. Soil cover is presented mainly by Cryosols, Gleysoils, Histosols and Entic Podzols with variation of permafrost table location about 70 v120 cm (Alekseev *et al.* 2017). Period of soil biological activity is estimated about 100–120 days (Abakumov and Alekseev 2017) which is the highest value for Siberian tundra.

Sampling plot No 1 was situated close to the Aksarka settlement (Fig. 1). The A1 0-9 AY soil sample represents a superficial layer of reclaimed soil located on

Table 1.

General characteristics of soil samples

Sample ID, depth [cm]	GPS coordinates	Lanвuse type /soil name	Reclamation practice	horizon type	color, Munsell color system
Y1	N66°51'50,7" E65°26'57,4"	quarry on the way from Chornaya Mt. to Kharp, Leptosol	abandoned heap of mine of sands	AY	10 R 5/1
Y2	N67°35'4,0" E68°18'49"	surroundings of Vylkato Lake, Leptosol	abandoned heap of mine of clays	AY	10 R 5/1
A1	N66°32'10,5" E67°47'12,2"	Aksarka reclamation plot, Technosols Transportic	reclaimed heaps of sands	AY	10 R 5/1
A2 (0–2)	N66°32'09,3" E67°47'02,3"	Aksarka reclamation plot, Cryosol Luvic Follic	mature larch forest	AY	10 R 5/1
A2 (2–30)	N66°32'09,3" E67°47'02,3"	Aksarka reclamation plot, Cryosol Luvic Follic	mature larch forest	CR	2.5 Y 7/1
A2 (30–80)	N66°32'09,3" E67°47'02,3"	Cryosol Luvic Follic	mature larch forest	G	10 G 6/1
YK (0–9)	N66°49'43,9" E65°36'40,8"	Surroundings of Chornaya Mt., Leptosol	abandoned heap of mine of chromatic quarry	AY	10 R 5/1
YK (9–32)	N66°49'43,9" E65°36'40,8"	Surroundings of Chornaya Mt., Techosol, transportic	abandoned heap of mine of chromatic quarry	C	

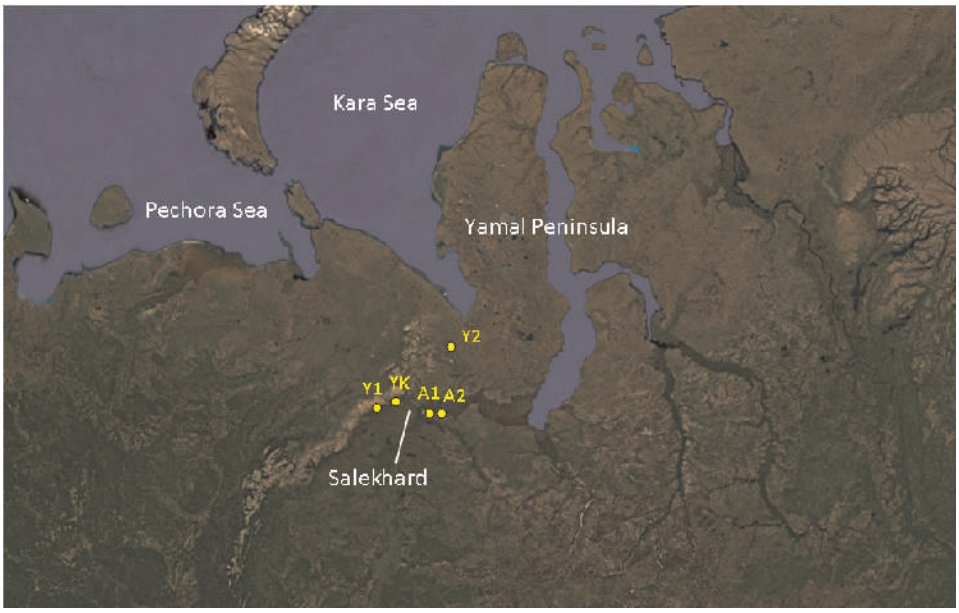

Fig. 1. The location of sampling plots.

the loamy textures heap of a former quarry, designated for mining of sandy-grave sediments for local construction purposes. Reclamation was done by a plantation of cereal plants, and this resulted in the formation of a dense soil cover. Next, three samples (A2 0-2 AY, A2 2-30 CR, A2 30-80 G) were collected from mature soil – Cryosol with features of podzolization in the top solum and features of gleyification in the suprapermafrost border. This soil under the larch was chosen as a reference benchmark soil. Also, three of the soils were samples from the surroundings of Chornaya Mt., where mines of chromitic ores are situated. The heaps of the mine are abandoned, covered by coarse stony entic soils with sparse vegetation. Only AY – gray humus horizons were sampled. The last sample (Y2 0-10, horizon AY) is located in the surroundings of Vylkatoi Lake; this quarry was used for exploitation of quaternary clays for road construction activity. Location of sampling plots was chosen not only according to their specificity as the objects, but also for logistic reasons: it was necessary to store soil samples in a frozen state, and that is not easy in case of transportation facilities in the tundra.

Methods

Soils for further routine analyses were grounded and sieved at 2 mm; the mineral samples were dried and sieved at < 2 mm, and the large root debris was picked out manually. Carbon and nitrogen content was determined with use of

C-H-N analyzer and data was converted to air-dried form. The pH values were determined in a routine way in water and calcium chloride suspensions. Exchangeable forms of soil acidity were determined by titration $CaCl_2$ and CH3COONa extracts by 0.1 M NaOH. Particle size distribution and texture class were determined using pipette-by Kaczynski with pyrophosphate peptization of microaggregates (Rastvorova *et al*. 1998).

Soils for DNA extraction were not ground before analyses. Samples were frozen in the field and transported to the laboratory. DNA was extracted from 0.2 g of soil using the PowerSoil DNA Isolation Kit (Mobio Laboratories, Solana Beach, CA, USA), which included a bead-beating step, according to the manufacturer's specifications. Homogenization of the samples was performed using Precellys 24 (Bertin Corp, USA) at 6.5 m/sec, twice for 30 s. The purity and quantity of DNA were tested by electrophoresis in 0.5 × TAE buffer on 1% agarose. DNA concentrations were measured at 260 nm using a SPECTROStar Nano (BMG LABTECH, Ortenberg, Germany). The average DNA yield was 2–5 µg DNA, with concentrations between 30 and 50 ng/µl. The purified DNA templates were amplified with universal multiplex primers F515 5′ - GTGCCAGCMGCCGCGGTAA-3′ and R806 5′ -GGACTACVSGGG-TATCTAAT-3′ (Bates *et al.* 2011) 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 expected length of the amplification product was 400 bp. Sequencing of the amplicon libraries was carried out using Illumina MiSeq in the Centrum 'Genomic Technologies, Proteomics and Cell Biology' (All-Russia Research Institute for Agricultural Microbiology). The raw sequences were processed using QIIME (Caporaso *et al.* 2010). Preliminary processing of the raw reads was performed using TRIMMOMATIC software (Bolger *et al.* 2014). To reduce sequencing errors, the multiplexed reads were first filtered for quality and grouped according to barcode sequences. Sequences were omitted from the analysis if they were less than 200 bp, had a quality score of less than 25, contained uncorrectable barcodes, primers, ambiguous characters or a homopolymer length equal to or greater than 8 bp. All non-bacterial ribosomal sequences and chimeras were also removed from the libraries. Chimeras were removed by using chimera_slayer.py script, incorporated in QIIME. In total, 1 023 728 sequences were obtained with an average of 33 023 sequences per library. The dataset was subjected to the normalization procedure, resulting in 16 365 sequences per sample. Similar sequences were clustered into operational taxonomic units (OTUs) with a minimum identity of 97% using de novo and closed reference algorithms. A representative set of sequences was chosen by selecting the most abundant sequence from each OTU. Representative sequences from each OTU were subjected to an RDP naïve Bayesian rRNA Classifier (Wang *et al.* 2007) with a confidence level of 80% and aligned using a PyNast algorithm and Greengenes database (DeSantis *et al.* 2006). Aligned sequences were used to build a distance

matrix with a distance threshold of 0.1 and phylogenetic tree necessary for downstream analysis.

To compare microbial communities, alpha- and beta-diversity analyses were performed. To estimate alpha diversity, the indices for richness (observed species, Chao1) and evenness indexes (Faith's index, Shannon evenness) were calculated. The t-test was performed to verify the observed differences. For beta-diversity, the weighted Unifrac metric (Lozupone and Knight 2005) was used to calculate the amount of dissimilarity (distance) between bacterial communities to be compared. The results were presented in PCoA analysis using "Emperor" implemented in QIIME. All estimates were measured for the normalized data (normalization was carried out to the smallest number of sequences present in the sample). With an aim to reveal a correlation between beta-diversity of samples studied (both weighted unifrac and unweighted unifrac) and factors of nutrient support (C, N concentration, C/N ratio, pH and others) Mantel test was performed (Lauber *et al.* 2009).

Relative abundances of bacterial and fungal small subunit rRNA gene copies were analyzed by quantitative PCR (qPCR), as previously described (Pershina *et al.* 2015).

To identify environmental parameters that affected the structure of soil microbiomes, the Mantel test with 999 permutations was performed using unweighted unifrac distance matrices for samples.

The abundances of OTUs were compared between samples by calculating the median relative change values for all groups of replicates. A positive median indicates an increase in abundance, whereas a negative median can be considered as evidence for a decline in abundance. A basic permutation test was used to infer significance, whereas a Beta diversity-like resampling approach was applied to test the stability of median estimates.

All sequences were deposited to the SRA (NCBI) within the dataset: SUB4606526.

Results

Soil general characteristics. – The soils of the former mines are classified as weakly developed and categorized as Leptosols and Regosols according to the low bioclimatic potential of the regions and exposition of coarse friable overcompacted grounds on the surface of heaps of former mines. In these conditions, the soil is represented by a weakly developed humus horizon of the gray type – AY, sublayed by a transitional AC layer and C horizons, followed by R – a solid massive rock horizon. Soils of the quarries are more aerated and insolated than soils of the mature landscapes, the last demonstrated redoximorphic features, connected with over moisturizing and stagnification (Table 2).

The investigated soils showed a low content of organic carbon and total nitrogen. All of the investigated soils were slightly acid with well-pronounced

Table 2.

Basic chemical soil characteristics and clay content

Sample ID	TOC, g/kg	N, g/kg %	C/N	pH H20	pH CaCl2	Exchangeable acidity, CMOLP+/kg	Hydrolithical acidity, CMOLP+/kg	Basal respiration, mg CO/kg^{-1} h^{-1}	Coarse fraction, %	Clay, %
Y1	40.7	2.2	21.57	5.15	4.47	0.20	0.30	0.03	12	14
Y2	4.1	4.0	10.89	5.76	5.12	0.20	0.20	0.03	7	14
A1	3.8	4.0	9.56	6.63	6.21	0.05	0.07	0.02	7	16
A2(0-2)	4.2	4.0	9.93	5.64	5.23	0.40	0.60	0.03	5	12
A2(2-30)	4.3	5.0	8.80	6.31	6.12	0.30	0.40	0.02	6	16
A2(30-80)	3.8	5.0	8.43	6.45	6.23	0.10	0.15	0.01	9	15
YK(0-9)	6.9	6.0	12.01	5.98	5.34	1.10	1.20	0.02	35	8
YK(9-32)	2.8	4.0	7.70	5.34	4.98	0.60	1.00	0.01	45	14

Fig. 2. The sampling plots pictures : 1- Y1, 2 - Y2, 3- A2, 4 -YK..

forms of exchangeable acidity, connected with the organic part of soil colloids (acidity in CH_3COONa extracts).

Alpha-diversity of soil microbiomes. – To evaluate the alpha-diversity of the soil microbiomes, several indices for species richness and evenness were calculated (Table 3). The values for the library coverage estimator ranged from

Table 3.

Alpha-diversity parameters of the soil samples

Sample ID	Shannon Index	Faiths' Index	Chao1	Number of OTU*	% coverage
Y1	9.7±0.2	203.0±8.5	3807.7±272.8	2094.5±125.3	55.0
Y2	8.4±0.5	174.3±12.1	3815.3±432.0	1819.5±165.9	47.7
A1	8.7±0.5	123.4±5.6	2661.7±290.7	1594.0±134.0	59.9
A2(0-2)	9.0±0.1	175.1±1.2	3012.1±291.1	1643.5±38.7	54.6
A2(2-30)	7.9±0.1	138.1±7.6	2134.7±266.8	1229.5±83.0	57.6
A2(30-80)	8.7±0.2	170.4±4.9	2891.4±154.4	1645.7±88.0	56.9
YK(0-9)	9.4±0.2	218.0±9.6	3835.8±286.4	2044.5±111.8	53.3
YK(9-32)	9.1±0.3	191.4±7.7	3095.8±265.8	1741.3±116.8	56.2

* OTU – operational taxonomic units

47.7% to 59.9%, meaning that approximately half of the OTUs were covered during the sequencing effort. The microbiomes of the abandoned heaps (Y1, Y2, YK) differed significantly from those of the undisturbed soils A2 and technosols A1, having the highest levels for species richness, phylogenetic diversity, and the Shannon index. The cryosolic horizon of the mature soil (A2 (2-30)) showed the lowest values of biodiversity among all compared samples, whereas the same horizon in disturbed soil of the quarry YK did not show this pattern, retaining the relatively high values of all indices.

Real-time PCR. – The tendencies measured during the analysis of alpha-biodiversity parameters were also found in a quantitative analysis of soil microbiomes (Fig. 3).

In mature soil, fungi amounts decreased in the cryosolic zone compared to the upper and subcryosolic soil layer; whereas bacterial counts were similar, even in the cryosolic horizon. This soil showed the lowest values for the Archaea abundance; hence, the tendency of soil stratification was unexpressed for these prokaryotes. The abandoned areas at A2 showed the same pattern in microbial counts, demonstrating the pronounced decrease in the number of fungi and repeatability in bacterial counts in the subsurface horizon (A2(2-30)). Samples A1 showed the highest values of microbial counts, especially for the Archaea. Compared to the mature soil, bacterial counts for the abandoned areas Y1 and Y2 were substantially higher, whereas the fungi reached their maximal counts in the upper horizon of the A2 samples.

Beta-diversity of soil microbiomes. – Analysis of beta-diversity is illustrated by the PCoA plots in Fig. 4. All samples formed separate clusters. Y2 mining sites showed the highest similarity to the mature soil samples. Reclamation practice had the strongest impact on the microbiome structure, separating A1 samples from other sites (Fig. 4).

Influence of the chemical properties of soil. – In attempt to reveal a correlation between beta-diversity of our samples (both weighted unifrac and unweighted unifrac) and nutritional factors (C, N concentration, C/N ratio, pH and other) Mantel test was performed (Lauber *et al*. 2009). According this data, carbon and nitrogen concentrations, also as pH values, are moderately correlate ($R = 0.31$) with beta-diversity, calculated via unweighted unifrac. Nitrogen level reveal small correlation ($R = 0.22$) with weighted unifrac beta-diversity, while a carbon level hadn't this correlation at all ($R = 0.02$) (Table 4). Our data correspond well with previously obtained (Lauber *et al*, 2009). These data also confirmed our suggestion that beta-biodiversity clasterization of samples (Fig. 4) is connected mainly with soil fertility levels, which is suspected especially for initial stages of soil formation, while the intensity of biogenic—abiogenic interactions is the highest during the history of soil development.

Taxonomic analysis of soil microbiomes. – The taxonomic analysis of the soil microbiomes revealed 50 bacterial and archaeal phyla, among which Proteobacteria (24% on average), Actinobacteria (18%), Acidobacteria (17.4%), Chroloflexi (9.7%), Gemmatimonadetes (6.5%), Verrucomicrobia (5.6%), Planctomycetes (4%), Bacteroidetes (3.9%), AD3 (2.7%) and Nitrospirae (1.1%) constituted the majority (more than 95% of sequences in the amplicon libraries; Fig. 5).

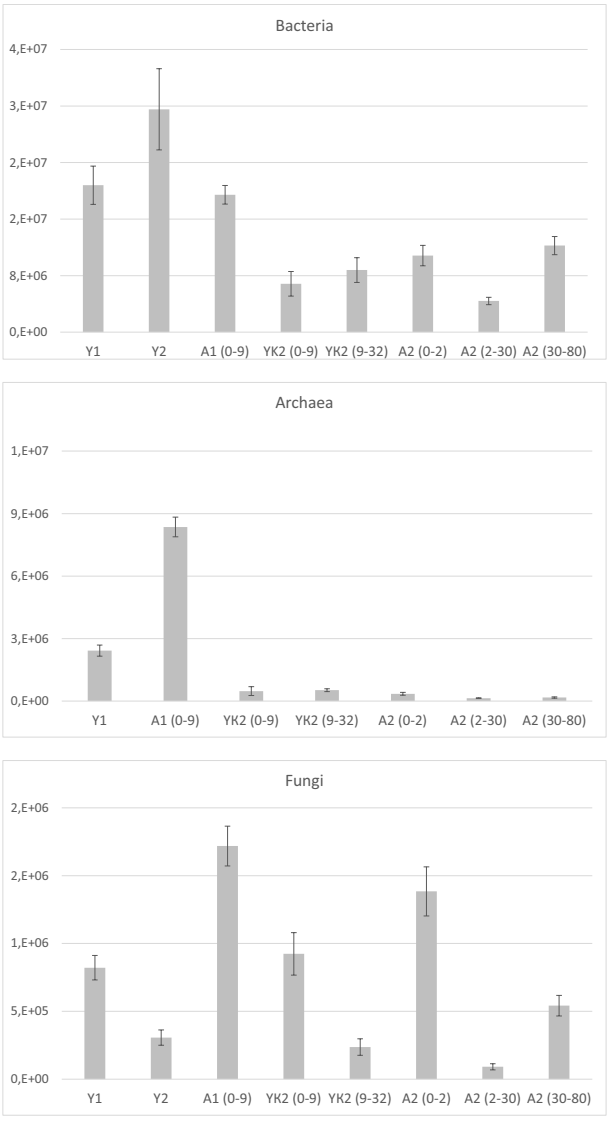

Fig. 3. Quantity of bacterial, archaeal and fungal operons in studied soil samples, acquired by Real-time PCR.

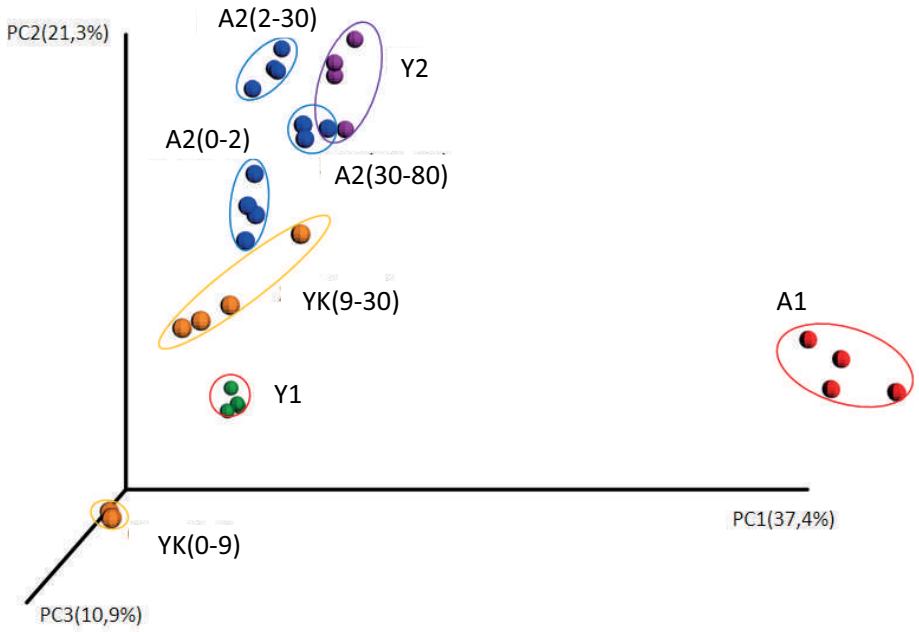

Fig. 4. Beta-diversity assessed by weighted and unweighted unifrac. PCoA for axis 1–2 and 1–3 are shown to see different dimensions.

Table 4.

Analysis of the correlation of the beta-diversity estimates with the physico-chemical properties of soil

Parameter (total, %)	R*	p-value**	R*	p-value**
Unweighted unifrac***			Weighted unifrac****	
N	0.32	0.005	0.22	0.046
C	0.31	0.011	0.02	0.066
C/N	0.27	0.356	0.04	0.871
pH	0.31	0.110	0.21	0.346
Coarse fraction (%)	-0.04	0.895	-0.02	0.924
Clay (%)	0.01	0.995	0.18	0.481

* R – correlation coefficient, ** p-value – significance ($p \leq 0.001$), *** unweighted (unweighted unifrac – beta-diversity matrix with qualitative counts of OTUs), **** weighted (weighted unifrac - beta-diversity matrix with quantitative counts of OTUs)

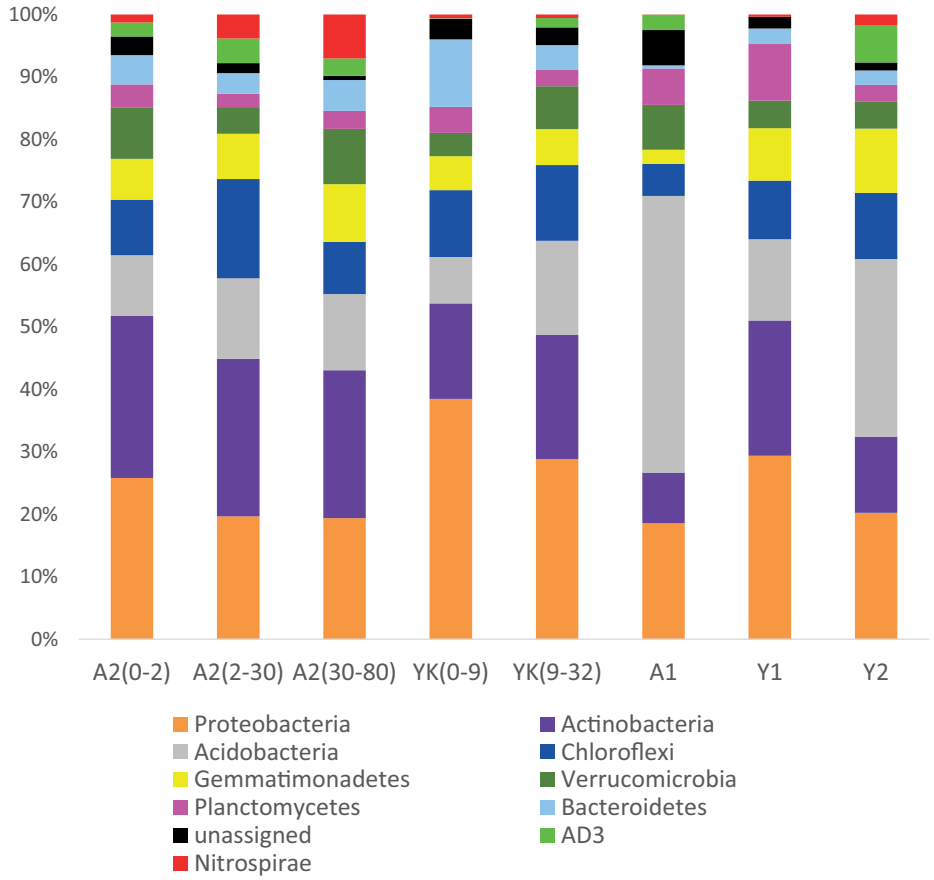

Fig. 5. Taxonomic composition of soil microbiome.

Archaea were represented by the phyla Crenarchaeota (0.2%), Euryarchaeota (0.1%) and Parvarchaeota, which were detected in the upper horizon (0–9) of the YK sample. The number of unidentified sequences reached 2.7% on average. Proteobacteria dominated in at least all soil samples, except for the sample A1, where it was substituted by the phylum Acidobacteria.

The soil microbiome of post-mining soils is dominated by bacteria from the phyla Acidobacteria (family Koribacteraceae, order RB41), Actinobacteria (families Intrasporangiaceae and Gaiellaceae), Chloroflexi (class Ellin6529), Gemmatimonadetes (order N1423WL), and Verrucomicrobia (family Chthoniobacteriaceae) (Fig. 6).

The microbiome of reclaimed soils from site A1 differs greatly from the other soils, mainly due to the increase in the abundance of Acidobacteria. In this soil, about 50% of all OTU was occupied by acidobateria taxa from the families Koribacteraceae and Acidobacteriaceae and order Ellin 6513. A significant increase in the proportion of the Proteobacteria from the family Syntropho-

Phyla	Class	Order	Family	Genus	A2 (0-2)	A2 (2-30)	A2 (30-80)	YK (0-9)	YK (9-32)	A1	Y1	Y2
Acidobacteria		11-24	NA	NA								
		RB41	NA	NA								
	[Chloracidobacteria]	RB41	Ellin6075	NA								
	Acidobacteria-6	iii1-15	NA	NA								
	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	NA								
			Koribacteraceae	NA								
	DA052	Ellin6513	NA	NA								
	iii1-8	32-20	NA	NA								
		DS-18	NA	NA								
	Solibacteres	Solibacterales	NA	NA								
			Solibacteraceae	Candidatus Solibacter								
Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA								
			EB1017	NA								
	Actinobacteria	Actinomycetales	Intrasporangiaceae	NA								
			Microbacteriaceae	NA								
			Micrococcaceae	NA								
	MB-A2-108	0319-7L14	NA	NA								
	Thermoleophilia	Gaiellales	Gaiellaceae	NA								
		Solirubrobacterales	NA	NA								
AD3	ABS-6		NA	NA								
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA								
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	NA								
	Ellin6529	NA	NA	NA								
	Gitt-GS-136	NA	NA	NA								
	Ktedonobacteria	Thermogemmatisporales	Thermogemmatisporaceae	NA								
	P2-11E	NA	NA	NA								
	S085	NA	NA	NA								
Gemmati-monadetes	Gemm-1	NA	NA	NA								
	Gemmatimonadetes	N1423WL	NA	NA								
Nitrospirae	Nitrospira	Nitrospirales	0319-6A21	NA								
Planctomycetes	Phycisphaerae	WD2101	NA	NA								
	Planctomycetia	Gemmatales	Gemmataceae	NA								
		Pirellulales	Pirellulaceae	NA								
		Planctomycetales	Planctomycetaceae	Planctomyces								
Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA								
			Bradyrhizobiaceae	NA								
			Hyphomicrobiaceae	Rhodoplanes								
	Betaproteobacteria	NA	NA	NA								
		Burkholderiales	Comamonadaceae	NA								
				Delftia								
			Oxalobacteraceae	NA								
		Ellin6067	NA	NA								
		IS-44	NA	NA								
		MND1	NA	NA								
	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter								
		Myxococcales	NA	NA								
		Syntrophobacterales	Syntrophobacteraceae	NA								
	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA								
			Xanthomonadaceae	NA								
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	NA	NA								
	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	DA101								
	Opitutae	Opitutales	Opitutaceae	Opitutus								
NA	NA	NA	NA	NA								

Fig. 6. Heatmap of the most abundant phylotypes for samples.

bacteraceae and bacteria from family Thermogemmatisporaceae (phylum Chloroflexi) was also detected for the A1 sample. It is worth noting that some taxa (mainly from the phyla Acidobacteria and Actinobacteria) accumulate in the

Fig. 6 continued

Phylum	Class	Order	Family	Genus	A2	YK	A1	Y1	Y2
Acidobacteria	[Chloracidobacteria]	RB41	NA	NA					
	Solibacteres	Solibacterales	NA	NA					
			Solibacteraceae	Candidatus Solibacter					
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	NA					
	Thermoleophilia	Gaiellales	Gaiellaceae	NA					
				NA					
		Solirubrobacterales	Conexibacteraceae	NA					
			Patulibacteraceae	NA					
Chloroflexi	Ellin6529	NA	NA	NA					
Gemmati-monadetes	Gemm-1	NA	NA	NA					
	Gemmatimonadetes	N1423WL	NA	NA					
			NA	NA					
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	NA					
				NA					
		Pirellulales	Pirellulaceae	A17					
				A17					
		Planctomycetales	Planctomycetaceae	Planctomyces					
Proteobacteria	Alphaproteobacteria	Ellin329	NA	NA					
		Rhizobiales	Bradyrhizobiaceae	NA					
			Hyphomicrobiaceae	Rhodoplanes					
		Rhodospirillales	Rhodospirillaceae	NA					
	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA					
				Delftia					
			Oxalobacteraceae	NA					
				NA					
		Ellin6067	NA	NA					
			NA	NA					
		IS-44	NA	NA					
		SC-I-84	NA	NA					
	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter					
	Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella					
		Legionellales	Coxiellaceae	Aquicella					
		Oceanospirillales	Halomonadaceae	Halomonas					
		Xanthomonadales	Xanthomonadaceae	Stenotrophomonas					
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Chthoniobacter					
				DA101					
	Opitutae	Opitutales	Opitutaceae	Opitutus					

cryogenic (subsurface) horizons of the soil samples A2 and YK, particularly the uncultivated members of the orders 11-24, RB41, 32-20 and 0319-7L14 (Fig. 6).

To find the ubiquitous bacteria among the analyzed soil samples, the core microbiome was identified for the upper soil layers (Fig. 7). This mainly consisted of the bacteria with low abundance values (rarely exceeding 1%). The major taxa in the core microbiome were Actinobacteria from the family Intrasporangiaceae and bacteria from the order N1423WL (phylum Gemmati-

monadetes) (Fig. 7). It is evident that reclamation practice results in intensive changes of soil microbiome in comparison with not reclaimed, e.g. abandoned lands. This fact should be taken into account in future investigations, related with elaboration of reclamation practices in the Arctic environments.

Discussion

The soil cover of Yamal has been investigated in only a few studies, which have been devoted mainly to natural soils (Alekseev *et al.* 2017; Matyshak *et al.* 2017a, b). In the papers mentioned classical pedological researches has been conducted, but not modern microbiological studies, based on new generation sequencing technologies. In this study, using a high-throughput new generation sequencing approach, the composition of the Yamal soil microbiome was investigated. Previous studies in this region were restricted by application of conventional microbiological methods (Kirtsideli *et al*., 2014; Vlasov *et al*. 2014, 2016, 2017; Abakumov *et al*. 2017).

The investigated soils showed a low content of organic carbon and total nitrogen, which was caused by a short period of soil regeneration. Soil regeneration ability/rate is considered very slow for tundra and forest-tundra zones relative to southern environments (Archegova *et al.* 2012), which accounts for the slow accumulation of biogenic elements in soils of former mines. The second reason is that in mature zonal soils organic carbon accumulates mainly in the form of histic layers or raw humus horizons. Only one soil sample (Y1) showed increased organic matter content, and this could be caused by the accumulation of the fine organic detritus type of soil organic matter, which cannot be separated from the fine mineral earth.

The level of basal soil respiration was low in all of the samples, which indicates the low level of soil microbiological activity. All of the soils contain a clay fraction, which is important for water retention capacity.

The physical properties of postmining soils differ greatly from those of natural Cryosols or Stagnosols.

The highest total content of bacteria, archaea, and fungi was revealed for soils of sandy (Y1) and clay (Y2) texture heaps and reclaimed soils close to the Aksarka settlement (A1). These values were essentially lower in natural soil A2 and the soil of abandoned heap YK of coarse stones of chromatic mines close to Chornaya Mt. Increased content of all three functional groups of micro-organisms in soils of Y1, Y2, and (reclaimed) A1 soils could be related to the favorable hydrophysical regime of those soils relative to natural soil with pronounced features of stagnification. At the same time, the low abundance of all groups of microorganisms in soils of abandoned heaps of Chornaya Mt. could be related to the toxicity of the soil matrix and low water-holding capacity due to a high coarse fraction content. Reclamation practice, expressed in covering of

soil by organic matter containing ground and planting of *Calamagrostis officinale*, results in a sharp increase of bacteria, archaea, and fungi counts. At the same time, reclaimed soils scored lower values in the Faith's and Shannon indexes relative to soils of abandoned mines. This indicates the formation of a transitional type of microbial community with the dominance of copiotrophic organisms in reclaimed lands.

The taxonomic composition at the A1 site differs greatly from the other soil samples. This site was dominated by acidobacteria, particularly from the families Koribacteriaceae and Acidobacteriaceae, which tend to be ubiquitous bacteria for the acidic type of soil and could also be found in warm soil environments in the temperate climatic zones (Lauber et al. 2009; Pershina et al.2018). The objects of investigation are located in relatively warm and humic part of Yamal Peninsula, so the abundance of Acidobacteriaceae could be expected here, also the presence of essential portion of organic remnants in the topsoil layers can be a reason of theses phylum of bacteria domination.

The presence of some abundant taxa might indicate the high rates of greenhouse gas emissions in the described site, particularly bacteria belonging to the Syntrophobacteraceae family. The sulfate-reducers are probably involved in methane-cycling (Liu *et al.* 2018) and are reported to be typical for permafrost environments (Gittel *et al.* 2014).

Comparison of the disturbed and mature soils revealed a significant increase in the number of bacteria in the soil subsurface (material of the horizons YK). This indicates the absence of stratification and restoration of the mature characteristics. Under this scenario, soil bacteria might be supplied with comfortable aeration conditions, as well as various substrates that were mixed during the quarry formation. All these factors favor the growth of the copiotrophic groups of bacteria. At the same time, there are some similarities between the cryosolic layer of the mature soils and the subsurface horizon of the YK sample, including the presence of the Actinobacteria (particularly from the families Intrasporangiaceae and Gaiellaceae), bacteria from the phylum Chloroflexi and unidentified betaproteobacteria, which have been reported to be typical microbial taxa for permafrost environments (Jansson and Taş 2014). The increase in the amount of these bacteria in YK and their low abundance rates in other disturbed sites illustrate the tendency of rehabilitation of this soil to the initial state. So, the presence of the phyla could be used as indication of the soil development rate under the implementation of reclamation practice.

Multiple taxa have been reported as dominant groups in previously described cold environments, including alpine soils. Particularly, Wu *et al.* (2017) reported Chthoniobacteraceae, Thermogemmatisporaceae, Ellin 6513, Koribacteriaceae, and Gaiellaceae to be the dominant groups at high elevation sites. Some taxa, particularly Gemmatimonadetes from the order N1423WL and *Chloroflexi* bacteria from the order Gitt-GS-136, were also found among the dominant part of the community in the similar marine affected permafrost environment

(Mitzscherling *et al.* 2017). Taxonomy composition of soils of post mining sites is typical for cold and humid environments, but it is different from the biodiversity of natural topsoils of the mature landscapes.

Conclusion

This study is the first attempt to describe microbial communities in the Yamal Peninsula region by use of modern molecular methods for qualitative and quantitative analysis of biodiversity. It was shown that mining activity leads to substantial changes in the microbial composition of the cryogenic soil environments, particularly in the case of reclamation practice. Reclamation leads to an increase in the amount of the specific groups of bacteria, which normally function as a minor component in the permafrost soils. This first attempt of biodiversity for mining and post mining environments should be continued in future for better understanding of self-restoration mechanisms of the microbial community in severe conditions of the merged part of forest tundra in North-West part of Western Siberia. Data on microbiological drivers of soil restoration should be taken into account for future elaboration and implementation of reclamation practices in areas of distribution of permafrost-affected soils.

The mature soil of the Yamal Peninsula exhibits many common bacterial taxa with the previously described permafrost environments cataloging the species inhabiting this specific ecological niche opens new opportunities for the future research.

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