Research Article

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Microbiome of abandoned agricultural and mature tundra soils in southern Yamal region, Russian Arctic

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Abstract: Soil microbiome plays a significant role in the development of the soil profile and the implementation of key soil biochemical processes. Observed Arctic warming creates significant environmental risks, since permafrost soils contain a huge amount of organic matter, which is in potential risk to be released in the form of greenhouse gases. This work is aimed to investigate the microbiomes in soils of mature tundra and anthropogenically affected areas of the Yamal region using a high-throughput sequencing approach. The taxonomic analysis of the soil microbiomes revealed 33 bacterial and archaeal phyla, among which the dominant were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroides*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Patescibacteria*, *Plantomycetes*, *Thaumarchaeota*, *Verrucomicrobia*, and *WPS-2*. The analysis of alpha and beta diversities revealed that undisturbed soil is completely different from anthropogenically affected ones in terms of microorganism biodiversity. Soils of the urban zone of Salekhard were found to be different with regard to the number of operational taxonomic units and beta diversity. Herein the microbiome in the upper and lower layers of soil developed under strong technogenic influence (M19) was unequal both in quality and in quantity. At the same time, soils developed in the recreational zone of Salekhard showed less differentiation profile of microbiome (former crop field and former vegetable garden soils). Microbial communities in the mature

tundra soil showed less differentiated microbiome composition.

Keywords: Yamal region, microbiome, tundra, soil, high-throughput sequencing

1 Introduction

According to official sources, 30–40 million hectares of arable lands have now been removed from agricultural use in Russia and are not used. They are transferred into the state of abandoned lands and transformed under the influence of natural and anthropogenic processes of soil formation, soil's self-development and degradation, forest growth, and waterlogging (Walker et al. 2009; Lyuri et al. 2010; Ivanov et al. 2015a,b). In recent decades, the Earth's surface has been considerably altered by human, resulting in noticeable change patterns in land cover (Kandissounon et al. 2018), which has consequent effect on the water cycle (Obianyo 2019).

Many regional governments of northern regions view the increasingly favorable growing conditions as an opportunity to manage agricultural expansion (Desyatkin et al. 2012; Ivanov et al. 2015a,b; Hohle et al. 2016; Chapagain 2017; Government 2017). For example, Siberia is currently at $\leq 50\%$ of its estimated potential for food production, including recovery of formerly abandoned lands, and it is described as a dormant breadbasket (Swinnen et al. 2017; Elfermik and Schierhorn 2019).

Despite the extreme natural and climatic conditions, the presence of cold weather and the frozen state of soils for 8–10 months a year, the focal nature of industrial and economic development of the territory Yamalo-Nenets Autonomous Okrug (YANAO) provides sufficient opportunity to the population with the main agricultural crops cultivated in the Far North (vegetables, barley, and perennial herbs). An important factor in the development of agricultural production in YANAO is the improvement in the agropotential of natural and effective fertility of arable and abandoned soils in

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accordance with their ecological-genetic status and particle size distribution, taking into account hydrothermal climatic indicators (precipitation, warm period duration, amount of cold period precipitations, regional features of their soil genesis and metabolism). Back in 1933, academic I. G. Eichfeld, in the book *The Struggle for the Red North*, wrote, “It would be necessary to critically review all old installations with regard to agricultural possibilities in the Far North.” Based on the results, the normative yield of the main crops cultivated in the harsh conditions of the Far North will allow the cultivation of many varieties of vegetable crops, including nonviral potato seed material, which is in line with the main objectives set out in the Federal Scientific and Technical Programme for the Development of Agriculture for 2017–2025 (Ed. Resolution of the Government of the Russian Federation No. 996 of August 25, 2017).

The experience of successful agricultural practices, including vegetable and potato production in the Arctic zone, is available, as evidenced by the regular consideration of this issue at the Circumpolar Arctic Conferences, which have already taken place. These conferences are extremely poorly represented by analyses of farming work in the Far North in Russia. However, vegetable production is common in Greenland, so the mainstreaming of farming practices in YANAO is very relevant and likely. The Arctic region has already proved to be a good perspective for the development of organic production, and the advantages of northern farming are already used by the Scandinavian countries and Finland. Development of domestic horticulture and locally grown agricultural production should be prioritized when evaluating the necessary investments in specific region's agriculture. Emphasis should also be placed on the development of facilities for processing, storage, and realization of agricultural good.

Despite the relatively long history of Siberian and northern vegetable growing – about four centuries – growing crops in northern part of Russia has progressed little to date. The first references to the breeding of cabbages, onions, and cucumbers in the Tobol North (on the territory of the current city of Salekhard) are found in 1907 in the works of A. A. Dunin-Gorkavić. In 1932, the Yamal Zone Vegetable Experimental Station of the All-Union Lenin Academy of Agricultural Sciences was established in the city of Salekhard (since 1938 it has been renamed the Salekhard Complex Zone Experimental Station of the Institute of Polar Agriculture, Livestock and Fisheries). The station carried out experimental works in the field of crop production: development of agricultural activities, selection, and production of new valuable varieties of cereals, vegetables, and fodder crops until 2007, when scientific works were gradually phased out.

In YANAO, dozens of farms were engaged in farming; most of them ceased to exist, and soils and lands turned into an abandoned state. There are also a number of greenhouses, both active and abandoned, which present an interesting object for studying soil processes and fertility parameters in closed soil conditions.

For the residents of the Far North which is characteristic of short summers and very harsh, long winters with polar nights, fresh vegetable crops are urgently needed, given their high vitamin contents and the presence of other biologically active substances (Kotelina *et al.* 2018). When transporting vegetables from other regions, some valuable properties are lost. Therefore, the active cultivation of crops in the territory of YANAO is resumed again.

Previously, the microbiome of soils in agro landscapes of Yamalo-Nenets autonomous regions were not studied. Therefore, this study is aimed to investigate the abandoned agricultural lands as well as soils of current agricultural fields in terms of microbiology.

In this regard, the scientific novelty of this research is that for the first time in the Yamal region the amount of soil transferred to the abandoned state is critical for further sustainable existence of the agro-industrial complex. A comprehensive study of the microbiome is carried out at various stages of post-anthropogenic evolution. The use of a metagenomic approach in the study of soil genesis was published only in two previous papers (Gladkov *et al.* 2019; Abakumov *et al.* 2020). Since soil genesis is largely dependent on the physiological and metabolic activities of soil microbiota, its study is essential in understanding the general mechanisms of soil formation processes (Frouz *et al.* 2016). However, so far, this analysis has been carried out mainly using cultural methods of analysis, which allow only a small part of the microbial community (1–10%) to be taken into account, while most of the microbiocenosis consists of uncultivable forms, which can be accounted and studied only using a metagenomic approach.

Based on the analysis of the biodiversity of the community of microorganisms, it is intended to clarify and expand our knowledge of the taxonomic and functional structure of microbiota at various stages of pedogenesis (Bates *et al.* 2010; Frouz *et al.* 2016; Harantova *et al.* 2017). The use of metagenomic and bioinformatic approaches for investigation of soil biota become quite required in modern soil science and reclamation technologies. In Russia, such an integrated approach has not yet taken a proper place, so this study is the first domestic study of the microbiome of abandoned soils of agroecosystems. In addition, it is planned to carry out an evaluation and cost assessment of soil development in time, which will allow to reach the solution of the new application problem of agricultural science,

agroecology, and agricultural economy – monetization of ecosystem services of components of agroecosystems in connection with the need to introduce systems of effective environmental management into the agro-industrial complex.

Previously, Arctic abandoned soils were investigated mainly through classical methods of soil chemistry and physics (Archegova 2007; Alekseev et al. 2016; Alekseev and Abakumov 2018), with no metagenomic studies of the microbiome of northern soils conducted in exception of two recent works (Gladkov et al. 2019; Abakumov et al. 2020).

The following objectives were formulated:

- To conduct metagenomic studies of soils of agrocryogenic ecosystems for understanding the microbiome structure.
- To understand key drivers of soil formation under various agricultural practices.

2 Material and methods

2.1 Regional setting and soils

This study was conducted in different locations of the Salekhard (Yamal autonomous region, Russia; Table 1 and Figure 1). Site 1 mature tundra soil (G10) is located in the northern direction from Salekhard and presented by mature tundra without evidences of anthropogenic transformation. Site 2 is located on the experiment field of the former zonal station. Site 3 is attributed to the field for potato cultivation (the right bank of Shaitanka river). Site 4 is located in the “Angalsky mys” area near the existing cowshed.

The climate of Salekhard is characterized by severity and continentality. Throughout the year, relative humidity is high (70–90%). It is caused by low air temperatures and proximity to the cold waters of the Kara Sea. Annual precipitation is about 350–400 mm in the southern part. The annual evaporation rate is about 250 mm. The number

of days with snow cover is 233 per year. Winter lasts 7–7.5 months; the average temperature of January is –23 to 25°C. Spring is usually short (35 days) and cold, with a sharp change in weather, with frequent returns of cold and frost. The vegetation season consists of 70 days. Average temperature in the warmest month is +5°C. The average annual temperature is –5.8°C. Autumn is short, with a maximal volatility of the pressure gradient, an abrupt change in temperature, and frequent early frosts. The site is in a zone of excessive moisture.

The soil cover of natural environments in the surroundings of Salekhard is characterized by the predominance of Histic Gleysols and Aquiturbic Cryosols in hydromorphic positions of the landscape and podzols in autonomous positions (Alekseev et al. 2017; Alekseev et al. 2019; Ji et al. 2019). Soils in this region are characterized by the low fertility level (low amount of nitrogen, phosphorus, and potassium). They have a low cation exchange capacity, base saturation and acid intervals of pH, and highly exchangeable and hydrolytic acidity.

Soil diagnostics were performed using both the Russian soil classification system (RSCs) (Shishov et al. 2004) and the World Reference Base for Soil Resources (WRB; FAO 2014). However, issues of soil profile morphology and classification have been discussed more detailed in the scope of RSCs.

Objects of investigation are presented in Figure 2. Objects are presented by agricultural soils from various functionals zones of Salekhard and one soil from mature tundra.

RSCs divide soils affected by agricultural influence into two orders (both are referred to the trunk of postlithogenic soils). The first order is agrozems. It unites soils having topsoil consisting of an agrohorizon (humus agrohorizon, gray humus agrohorizon, peaty agrohorizon, or peaty-mineral agrohorizon). In the soil profile, such a topsoil should be changed by the natural diagnostic horizon (B) or parent material (C) very sharply. The authors of the classification system also notice that soils from the above-mentioned order can be developed in any conditions and any natural zone. The types of agrozems are determined by

Table 1: The main characteristics of investigated sites

Site	Geographical coordinates	Functional zone	Soil type (WRB/RSCs)
1	N 67°26′39,3″; E 67°22′35,7″	Mature tundra	Cambic podzol/podbur
2	N 66°31′02,3″; E 66°39′22,3″	Recreational functional zone (former crop field)	Spodic anthrosol/Al-Fe humic agrozem
3	N 66°32′56,8″; E 66°36′43,4″	Recreational functional zone (former vegetable garden)	Spodic anthrosol/Al-Fe humic agrozem
4	N 66°33′31,9″; E 66°34′07,2″	Recreational functional zone (urbanized ecosystem)	Spodic anthrosol/Al-Fe humic podzol

Figure 1: The map of the study area. Sites: 1 – Mature tundra, 2 – recreational functional zone (former crop field); 3 – recreational functional zone (former vegetable garden); 4 – recreational functional zone (urbanized ecosystem).

features of the agrohorizon and its combination with the underlying natural horizons.

The second order is Agroabrazems. Soils from this order are deprived of the topsoils and in some cases diagnostic (B) horizons of natural soil due to erosion, deflation, mechanical cutting, etc. The specific feature of Agroabrazems is the presence of a specific agroabraded horizon formed due to transformation of the diagnostic (B) horizon or the parent material (C). The types of Agroabrazems are determined by the horizon features underlying the arable horizons. In the studied soil profiles, we have not found any feature of Agroabrazems and agroabraded horizons.

It should be noticed that the character of the parent material described in studied soil profiles and that prevailed within the Salekhard city area (sandy material) was one of the main reasons for favorable agricultural using of land in the north of Western Siberia.

At the same time, in the WRB system, it is determined that only one reference soil group soil the soil affected by agricultural activities – anthrosols. This reference soil group is characterized by the soils that have been formed or heavily modified by the long-term human activities (addition of organic materials or household wastes, irrigation, cultivation, etc.). Anthrosols often can occur in association with a variety of reference soil groups. It should be noticed that these linkages have been mainly studied for nonpermafrost landscapes. So in the zones with a predominance of wetland soils such as fluvisols, gleysols, histosols, and podzols and presence of agricultural activity, terric anthrosols are usually observed.

Data on the soil chemical state have been published earlier (Alekseev and Abakumov 2018). Briefly, these soils are acids, with low total and nitrogen content in middle horizons of the profile and accumulation of these elements in the topsoil. These soils have increased portion of exchangeable nutrients. It was suggested that the presence of permafrost table and low biological activity during the abundance save the agrochemical state of the previously amended soils for longer time than in the case of leached soils (podzol) of more south (boreal) environments.

2.1.1 Metagenomic studies

2.1.1.1 DNA isolation

DNA was isolated using the MN NucleoSpin kit (MN, Germany). A Precellus 24 homogenizer (Bertin, USA) was used as a destructive mechanical action. The quality of the resulting preparation was checked electrophoretically (1% agarose gel; Bio-Rad, USA) and by PCR (Bio-Rad T100 Thermal Cycler).

2.1.1.2 DNA sequencing

DNA sequencing was performed via the Illumina MiSeq sequencing system (USA), with primers F515 (GTGCCAGCM GCCGCTAA) and R806 (GGACTACVSGGGTATCTAAT) for the V4 variable region of the 16s rDNA gene according to the manufacturer's manual.

1 - Mature tundra	2 - Recreational functional zone (former crop field)

3 - Recreational functional zone (former vegetable garden)	4 - Recreational functional zone (urbanized ecosystem)

Figure 2: Studied site and respective soil profiles (explanations given in Table 1).

2.1.1.3 Bioinformatic data processing

Sequence trimming was performed using the Trimmomatic program (Bolger et al. 2014). Forward and reverse reads

were combined using the *fastq-join* software of expression analysis (Aronesty 2013). The VSEARCH open-source tool (Rognes et al. 2016) was used to search and filter the chimeric sequences. Operational taxonomic units (OTUs)

were determined using the close reference method based on the SILVA database (Yilmaz et al. 2014; Rognes et al. 2016). OTUs, attributed as chloroplast/mitochondria, were removed. Alpha- and beta-diversity analyses were also performed using the QIIME package (Caporaso et al. 2010), and the mantel test from the QIIME package with 999 permutations.

2.1.1.4 Real-time PCR

For quantitative PCR, we used primers against the three groups of microorganisms: bacteria – EUB338 (ACTCCTACG GGAGGCAGCAG) and EUB518 (ATTACCGCGGCTGCTGG); archaea – ARC915f (AGGAATTGGCGGGGAGCAC) and ARC1059r (GCCATGCACCWCCTCT); and fungi – ITS1f (TCC GTAGGTGAACCTGCGG) и 5.8S (CGCTGCGTTCCTCATCG). To prepare the reaction mixture, the qPCRMix-HS SYBR kit (Eurogen, Russia) was used according to the manufacturer's instructions. A series of 10-fold dilutions of 16S (*Escherichia coli* and *Helicobacter pylori*) and ITS1 (*Saccharomyces cerevisiae*) fragments were used as standards. A CFX96 thermocycler (BioRad, Germany) was used to perform PCR using the cycling parameters: 95°C for 3 min, 40 cycles of 95°C for 20 s, 50°C for 20 s, and 72°C for 20 s.

For beta diversity, the weighted Unifrac metric (Lozupone and Knight 2005) was used to calculate the amount of dissimilarity (distance) between the bacterial communities to be compared. The results were presented in the principal component analyse (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).

3 Results and discussion

Data of alpha biodiversity of the soil microbiomes (Figure 3) showed that undisturbed soil is completely different from anthropogenically affected one in terms of microorganism biodiversity. Thus, the highest number of OTUs was in the soil of the former crop field (site 2). This can be due to the fact that soil was intensively involved in agricultural practices during many years, which is in line with the results from post-anthropogenic soils reported by Pershina et al. (2020). The lowest number of OTUs was found in the soil of the urbanized ecosystem (site 4). This could be due to the fact that this soil is located in a strongly technogenically affected part of the Salekhard city. The OTUs of the soil of the potato field (site 3) were intermediate between the former crop field and the urbanized ecosystem soils. No trends were observed in the distribution of number of OTUs within the soil profiles. It could be caused by the intensive processes of cryogenic mass exchange well pronounced in soils investigated (Ji et al. 2019). The Shannon indexes, normally, were higher in the top layer of mature soil, while they were not homogenously distributed within the profiles of anthropogenically affected soils. The values of Shannon indexes obtained are comparable with those in soils of abandoned post-anthropogenic soils of Yamal (Pershina et al. 2020) and Yakutsk (Abakumov et al. 2020) but higher while the topsoil of the remainder pastures of Yamal are compared (Laishev et al. 2020). As for Simpson indexes, they were comparable between all the soils investigated.

A total of 30 phyla of bacteria were identified in all soils studied. The main phyla of the soil microorganism in all samples investigated are as follows: *Proteobacteria* (23.41% on average), *Acidobacteria* (8.18% on average),

Figure 3: Alpha biodiversity of investigated soils.

Figure 4: Taxonomic composition of soil microbiomes.

Actinobacteria (15.45% on average), *Bacteroides* (7.79% on average), *Chloroflexi* (9.45% on average), *Cyanobacteria* (0.36% on average), *Firmicutes* (26.86% on average), *Gematimonadetes* (1.23% on average), *Patescibacteria* (0.46% on average), *Planctomycetes* (3.32% on average), *Thaumarchaeota* (0.73% on average), *Verrucomicrobia* (1.66% on average), and *WPS-2* (0.45% on average). They

accounted for more than 98% of all identified classes in each of the investigated soil samples. However, the taxonomical diversity of soil microbiomes (Figure 4) is completely different while one comparing mature and agricultural soils. Firmicutes were dominant in post-arable soils that are more dry and aerated (Chodak et al. 2015; Pajares et al. 2018), while Proteobacteria was more

Figure 5: Beta biodiversity of soils investigated.

typical for mature soils. The presence of Acidobacteria is typical for acids pedomicrobiomes and has been described previously by Pershina *et al.* (2020) and Chira *et al.* (2013) for polar and boreal ecosystems correspondingly. Acidobacteria has been previously shown to be strongly correlated with soil pH, which is in agreement with the findings of Jones *et al.* (2009). The study area is located in the southern Yamal, which is relatively warm and humid compared to the northern parts of the Yamal region; so the abundance of Actinobacteria could be explained by the presence of essential portion of organic remnants in topsoil layers. Some similarities between the mature tundra soil and soils from Salekhard are the presence of the Actinobacteria phyla, bacteria from the phylum Chloroflexi and WPS-2, which have been reported to be typical microbial taxa for permafrost environments (Jansson and Tas 2014). Fierer and Jackson (2006) did not succeed in classifying the Firmicutes phyla as either oligo- or copiotrophs. Firmicutes were previously described as one of the main drought-sensitive bacteria (Zhang *et al.* 2019).

Beta biodiversity (Figure 5) of the investigated soils was assessed by the weighted Unifrac metric (Lozupone and Knight 2005) to calculate the amount of dissimilarity (distance) between bacterial communities with all weighted, unweighted, and Bray–Curtis distance measures. All the samples formed separate clusters. At the same time, upper and lower layers from the urbanized ecosystem (site 4) form different clusters (in both weighted and unweighted Unifrac), while upper and lower layers from the mature tundra soil, former crop field (site 2), and former vegetable garden (site 3) soils can be clustered together. Nonmetric multidimensional scaling with Bray–Curtis distance showed that microbial communities of upper and lower layers in each mature tundra soil (site 1), former crop field, and former vegetable garden soil are clustered together, while urbanized ecosystem soil forms two different clusters in upper and lower layers.

Former crop field (site 2) and former vegetable garden (site 3) soils have similar bacterial communities along the soil profile, which suggests that bacterial community structures are quite the same in the recreational functional zone of Salekhard. This has been previously reported for post-anthropogenic soils (Pershina *et al.* 2020). At the same time, qualitative and quantitative structuring and differences in upper and lower layers of the microbial community reveal the role of anthropogenic disturbance in the urbanized ecosystem (site 4) soil. This could be caused by mechanical disturbance and mixing of soil layers due to the maintenance of the cowshed. Mature tundra soil shows the most similar microbial

community along the soil profile, which suggests that mature tundra soils are different from their urbanized analogues because of the more stable microbiome, which is in line with the previous research (Chodak *et al.* 2015; Pajares *et al.* 2018).

Numerous natural and anthropogenic factors influence soil fertility and microbial community and have to be considered when interpreting the soil microbiome parameters (Yan *et al.* 2015; Li *et al.* 2017). In our work, we discuss only some of them. However, climatic parameters (such as temperature, precipitation, and other climatic events), which can significantly affect the soil microbial community, probably do not have a significant effect on microbial diversity since the soil samples were collected from the same bioclimatic region and thus were exposed to similar weather conditions.

Arctic warming may significantly change the functional diversity of microbial communities in permafrost soils. At the moment, the deficiency of observation and analytical data on the microbiome of Arctic soils leads to the necessity to intensification of scientific research in this area. It is of particular interest the issues of investigation of mutual relationship between microbial diversity and environmental parameters associated with soil functioning, namely, nutrient dynamics controlled by microbiome, soil organic matter mineralization, and significance of changing environmental conditions for the soil microbiome.

4 Conclusions

This study is one of the first attempts to describe soil microbial communities in mature tundra and anthropogenically affected sites of the Yamal region by the use of modern molecular methods for the qualitative and quantitative analyses of biodiversity.

Of the 30 identified phyla, 13 explain more than 99% of all microbial diversity. This investigation reveals such most abundant phyla of soil microorganism in samples collected from natural and anthropogenically affected soils: *Firmicutes* (26.86% on average), *Proteobacteria* (23.41% on average), and *Actinobacteria* (15.45% on average). It was revealed that Firmicutes phyla are mostly abundant in post-arable soils, which are the driest and more aerated. Proteobacteria was mostly described in mature tundra soil, while the presence of Actinobacteria is explained by the location of the study area in a relatively humid part of the Yamal peninsula. The highest number of OTUs is in spodic anthrosol from

the former crop field, since this site was intensively involved in agricultural practices during last decades. At the same time, the lowest number of OTUs was found in the most technogenically affected part of the Salekhard city.

The analysis of beta diversity of microbial communities revealed that mature tundra soil shows less differentiated microbiome composition along the soil profile, while soils from anthropogenically affected areas of Salekhard could differ in microbiome composition when comparing upper and lower soil layers. The described soil microbial community in mature soil exhibits many common bacterial taxa for the Arctic region. So our results could be used as a vital contribution to the comprehensive study of the soil microbiome in Arctic soils. This, in turn, is crucial for better understanding of ecosystem responses to global climate change.

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