

## Microbiome of abandoned soils of former agricultural cryogenic ecosystems of central part of Yamal region

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### Abstract

Microbial activity plays a crucial role in the development and formation of soil properties. The active and abandoned agricultural soils in the Arctic zone represent a valuable resource that can play a crucial role in providing food security in the northern regions. The reuse of abandoned land for agriculture will reduce environmental risks in the context of a changing climate. Therefore, there is a need for monitoring studies to assess changes in soil parameters after long-term abandonment (taxonomic diversity, agrochemical and physico-chemical qualities). In the study, we evaluated the taxonomic diversity of the microbiome in abandoned (postagrogenic) and pristine soils of the Central part of the Yamal region. In the process of taxonomic analysis, more than 30 different bacterial and archaeal phyla were identified. The formation of a specific microbiome associated with anthropogenic influence in post-agrogenic sites has been shown. Most common types of soil microorganisms in samples collected from pristine and postagrogenic soils were *Firmicutes* (average 26.86%), *Proteobacteria* (average 23.41%), and *Actinobacteria* (average 15.45%). *Firmicutes* phylum was found mainly in the agrocenoses soils, *Proteobacteria* were mainly described in the mature tundra soils, *Actinobacteria* in humid conditions. An increase in diversity indices in postagrogenic soils was shown.

**Key words:** soils, Arctic, metagenomics, 16S rRNA Sequencing

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### Introduction

The Yamal-Nenets Autonomous Okrug (District) (hereinafter, YNAO) is a key support region of the Russian Federation in the Arctic. The zone is of particular inter-

est as an area where both (1) large-scale land development, reclamation, and uncontrolled conversion to fallow land and (2) subsequent removal in recent years have

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taken place. All this led to the formation of the chronosequences of soils with varying degrees of exposure to agrogenic factors. Operating and fallow agro-ecosystems are unique lands, which represent the models of development, degradation and in general – the evolution of components of terrestrial ecosystems in time and space. They reflect multidirectional dynamics of agrogenic impact during the 20<sup>th</sup> century on the territory of the Russian Federation. In the post-Soviet period, vast areas of agrolandscapes were transferred to fallow lands (Lyuri *et al.* 2010). It resulted in the transformation of soil cover and soil properties, and was often expressed in the degradation of soil fertility. On the other hand, fallow soils have become a potential acceptor of organic carbon. The latter aspect is extremely important for the verification of parameters of the carbon balance in dynamically developing anthropogenic ecosystems, including agrogenic ones.

All this has led to the formation of a great diversity of soils with varying degrees of past and continuing exposure to agrogenic factors. These natural-anthropogenic models can be used as a series of soil degradation and analysis of the dynamics of their microbial community development over time, including the main genetic markers and microbiome specificity and/or diversity. Post-agrogenic soils are much more fertile than the pristine soils of the region. Therefore, they might be considered a valuable natural resource. It is important to establish monitoring studies of their quantity and properties, including temporal changes in microbiome communities.

Monitoring of soils in the YNAO, suitable for growing organic vegetable crops is additionally relevant in the problem of solving modern food security of settlements. This importance is intensified by the fierce competition for the markets of agricultural products, especially in the zones of risky agriculture. Taking into account the vulnerability of YNAO in depen-

dence on external food supplies, it is necessary to determine the possible conditions for the development of territories to ensure food independence of the district with its own ecologically pure vegetable products. Monitoring of fallow soils is also associated with the reduction of potential ecological risks (disturbance of the mature soil cover and the resulting invasion of atypical microbiota in Arctic soils), since the reintroduction of fallow agrosols into agricultural rotation will reduce new soil disturbances, which is especially important in the cryolithozone in the climate change context.

Soil microbiome play a crucial role in formation and retaining of soil fertility and quality (De Mandal *et al.* 2019, Abakumov *et al.* 2020, Chen *et al.* 2020, Kui *et al.* 2021, Nikitin *et al.* 2022), which is especially important for severe conditions of agriculture in cryolithozone. The majority of agricultural lands in Russia is concentrated in the European part of the country (<sup>[1]</sup>Stolbovoy 2002). For these agrosols, only limited data have been published in terms of soil microbiome investigation (Nikitin *et al.* 2020, 2021). Only few results have been published on the soil microbiome for Europe either (Jenkins *et al.* 2017, Bell *et al.* 2020). At the same time, about 60% of Russia is presented by cryogenic ecosystems and cryolithozone (Kotlyakov and Khromova 2002). Soils have a specific thermic regime and productivity here. Nevertheless, cryogenic soils were intensively used in the agricultural practices during Soviet times. Many of them have been in an abandoned fallow state for about 30 years. Thus, they represent a good example of recent soil evolution in a post-agrogenic state (Giani *et al.* 2004, Kalinina *et al.* 2018). However, at the same time investigations of microbiomes of abandoned agricultural soils are quite rare. Soils of Russian Siberia represent so-called “hidden food basket” of Eurasia (Swinnen *et al.* 2017, Unc *et al.* 2021). Thus, they could play an important future role in the for-

mation of food security in the Northern Hemisphere. Studies of the microbiological properties of soils in the YNAO were conducted in 1960-2004 by scientists of the Yamal zonal experimental agrostation (Morgun and Abakumov 2019). Tikhanovsky studied the quantity and biomass of the main taxonomic groups of soil microorganisms (Tikhanovsky 2021). After 2004, no comprehensive studies of the microbiome in agricultural ecosystems of the YNAO have been conducted. In this regard, our 2019-2021 work is further updated.

Soils of the taiga and tundra in the YNAO are initially unsuitable for farming and their introduction into the agricultural practices implies a fundamental transformation of the soil profile and its physico-chemical, agrochemical and microbiological (especially changes in taxonomic diversity) properties (Morgun and Abakumov 2019, Tikhanovsky 2021, Nizamutdinov et al. 2022, Suleymanov et al. 2022). When

assessing the quality of abandoned and active agricultural lands it is necessary to conduct a comparative analysis of the transformations by comparing them with natural objects. It is necessary to answer the questions: Are the abandoned soils suitable for farming after a long downtime and how do they differ in microbiological, agrophysical and physico-chemical properties from the background soils of the region at present?

Thus, the main goal of our work was to evaluate a taxonomy diversity of soil microbiome in the case of mature and former agricultural soils of the central part of Yamal region. Following objectives were formulated: (1) to select representative soil examples in abandoned former agricultural ecosystems and in benchmark environments, (2) to evaluate key parameters of soil fertility and (3) to characterize soil microbiome using modern methods of soil metagenomics.

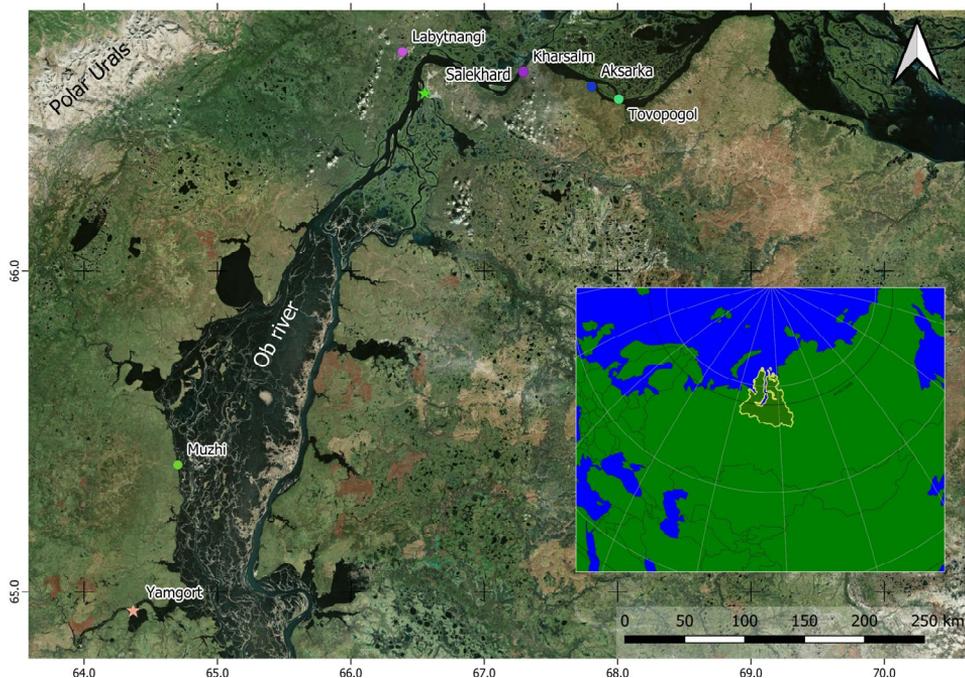
## Material and Methods

### *Regional settings*

The YNAO is characterized by extreme climatic conditions, since most of the region's territory is located above the Arctic Circle. The Arctic Ocean and The Polar Urals mts. contributes to the formation of a microclimate within the territory of YNAO. Thus, four zones are replaced from north to south in the region: arctic tundra, tundra, forest tundra and northern taiga. The climate is humid continental, with very cold winters and chilly summers. Precipitation is 220 to 400 mm per year, most of it in spring and summer. The average annual temperature is about 7°C.

Evaporation is low, so there is an excess of moisture almost everywhere (Forbes et al. 2009, Magnin et al. 2015).

We studied the soils sampled in the vicinity of Salekhard city (tundra zone) and the village of Yamgort (northern taiga zone) (Fig. 1). Both study sites are located in the cryolithozone, but differ in natural zones (northern taiga and tundra), therefore, we decided to examine the transformation processes of the mature soil microbiome under the influence of agriculture in these two key sites.



**Fig. 1.** Location of study areas.

The first site is an abandoned field in the city of Salekhard (Fig. 2). Cultivation has not been performed since 2019. Currently, it is a motley-grass phytocenosis (predominant plants - meadow horsetail (*Equisetum pratense*) and creeping couch (*Elytrigia repens*)). Soil name – Plaggic Podzol (Turbic).

The second site is the tundra in the vicinity of Salekhard. The main vegetation components are dwarf birch (*Betula nana*) and woolly willow (*Salix lanata*). Sedge (*Carex aquatilis*), cotton grass (*Eriophorum polystachion*), lingonberries (*Vaccinium vitis-idaea*), and various green mosses are found at the site as well. Soil name – Turbic Cryosol

The third site is an abandoned vegetable garden in the village of Yamgort. Dense grass cover consists of nettles (*Urtica dioica*), wheatgrass (*Elytrigia repens*), fescue (*Festuca ovina*), dandelion (*Taraxacum*

*officinale*), meadow horsetail (*Equisetum pratense*), plantain (*Plantago lanceolata*), clover (*Trifolium repens*) and willow-herb (*Chamaenerion angustifolium*). Soil name – Plaggic Podzol (Turbic).

The fourth site is a northern taiga near the village of Yamgort. Vegetation is formed mainly by larch (*Larix sibirica*) without grass cover. Green mosses and lichens on tree trunks are also present. Soil name – Plaggic Podzol (Turbic)

Soil samples for routine analyses were taken every 10 cm from the surface down to the depth of 30-100 cm. After sampling, they were placed in plastic bags and air dried after transportation (24°C, drying was carried out until the weight of the sample stabilized), then sieved through a sieve with a mesh diameter of 2 mm.

Samples for microbiological analysis were taken from topsoil horizons (0-20 cm). Sterility was maintained during

sample processing. Each sample was taken with sterile gloves and into sterile and sealed tubes. Samples were taken for each site in five replicates. After sampling, the

samples were immediately placed in a portable refrigerator at  $-20^{\circ}\text{C}$  and transported to the laboratory in a frozen condition.



Abandoned field in the city of Salekhard



Abandoned vegetable garden on the vicinities of Salekhard



Abandoned vegetable garden in the village of Yamgort

**Fig. 2.** Photos of sampling locations. *Note:* Left - vegetation cover, right - soil profile.

### *Soil analyses*

The pH values of soil solution were measured using a pH-meter-millivoltmeter pH-150MA (Antech, Belarus). Soil solution was prepared in the ratio of 1:2.5 with water or 1M calcium chloride (CaCl<sub>2</sub>) according to Black *et al.* (1965). Soil basal respiration was evaluated by measuring carbon dioxide (CO<sub>2</sub>) in sodium hydroxide. Incubation of CO<sub>2</sub> was conducted for 10 days in plastic sealed containers (Jenkinson and Powlson 1976). Soil organic carbon (SOC) content was determined by

the Tyurin method, based on the oxidation of soil organic matter with a mixture of potassium dichromate and concentrated sulphuric acid (Walkley and Black 1934, Bel'chicova 1965). The carbon of the microbial biomass was determined by fumigating the soil microorganisms with chloroform (ROTIDRY® ≥ 99,8 % (≤50 ppm H<sub>2</sub>O)) for 24 hours, followed by carbon determination in 0.5M soil extract in K<sub>2</sub>SO<sub>4</sub> (Vance *et al.* 1987).

### *DNA extraction and metagenomics research*

DNA was extracted from the soil and used to construct amplicon libraries of the 16S rRNA gene fragment. Sequencing and primary data processing were performed on an Illumina MiSeq (Illumina, Inc., USA) at the Centre for Genomic Technologies, Proteomics and Cell Biology (ARRIAM, Russia). DNA was isolated and sequenced using the developed technique described in Gladkov *et al.* (2019).

Data were processed using the pack-

ages in the R software environment (<sup>[2]</sup>R Team 2021) and QIIME2 (Bolyen *et al.* 2019) as described in Kimeklis *et al.* (2021). Taxonomic assignment of the phylotypes was determined using the Silva 138 database (Quast *et al.* 2013). The PERMDISP2 (vegan package) algorithm was used to analyze variation in beta diversity, and the reliability of differences was determined by the Tukey test (vegan package) (Anderson 2006).

## **Results and Discussion**

### *Soil Chemical and morphological characteristics*

The studied soils belonged to genetic types of podzols and cryosols (Plaggic Podzol (Turbic) – abandoned field in Salekhard, private garden and mature taiga in Yamgort, Turbic Cryosol – mature tundra in Salekhard). Soil of the abandoned field in Salekhard: Ap – 0-23 cm (post-agrogenic organomineral horizon); Bs,g1 – 23-31 cm (transitional illuvial-iron horizon with gleyic features); Bs,g2 – 31-41 cm (transitional illuvial-iron horizon with gleyic features and reductimorphic spots); Cg – 41-143 (parent material, alluvial sandy deposits underlain by permafrost).

Soils of pristine tundra near Salekhard: O – 0-13 cm (slightly decomposed organic matter of moss and tundra plants); Ag – 13-26 cm (coarse organic material with signs of gleyic colors and reductimorphic spots); Cg – 26-40 cm (heavy loamy materials with reductimorphic spots, underlain by permafrost). Soil of the abandoned vegetable garden in the village of Yamgort: Ap – 0-21 cm (post-agrogenic organomineral horizon); BCg – 21-41 cm (illuvial-humus-iron horizon, with humus plugs and redoximorphic spots, underlain by permafrost). Taiga soil near the village

of Yamgort: Oa – 0-4 cm (practically undecomposed forest litter consisting of moss and debris); Ar – 4-37 cm (illuvial-iron horizon with Iron-oxide nodules). The parent material underlying the soil profiles was constrained by permafrost. Previously, the morphological features of the abandoned soils of the Yamal region have been studied in more detail with the peculiarities of the structure of these soils you can see in (Alekseev and Abakumov 2018, Abakumov et al. 2020, Nizamutdinov et al. 2021, 2022; Suleymanov et al. 2022).

Typical tundra and northern taiga soils are acidic (pH < 6), which makes it necessary to regulate the acid/alkaline regime when involving them in agriculture (Alekseev and Abakumov 2018, Tikhanovsky 2021). However, even in the case of previous meliorative measures, we studied post-agrogenic soils are characterized by low values of pH, which increases gradually with depth (Fig. 3). The highest acidity, both actual (pH H<sub>2</sub>O) and potential (pH CaCl<sub>2</sub>), was recorded in the topsoil, humus-accumulative horizons.

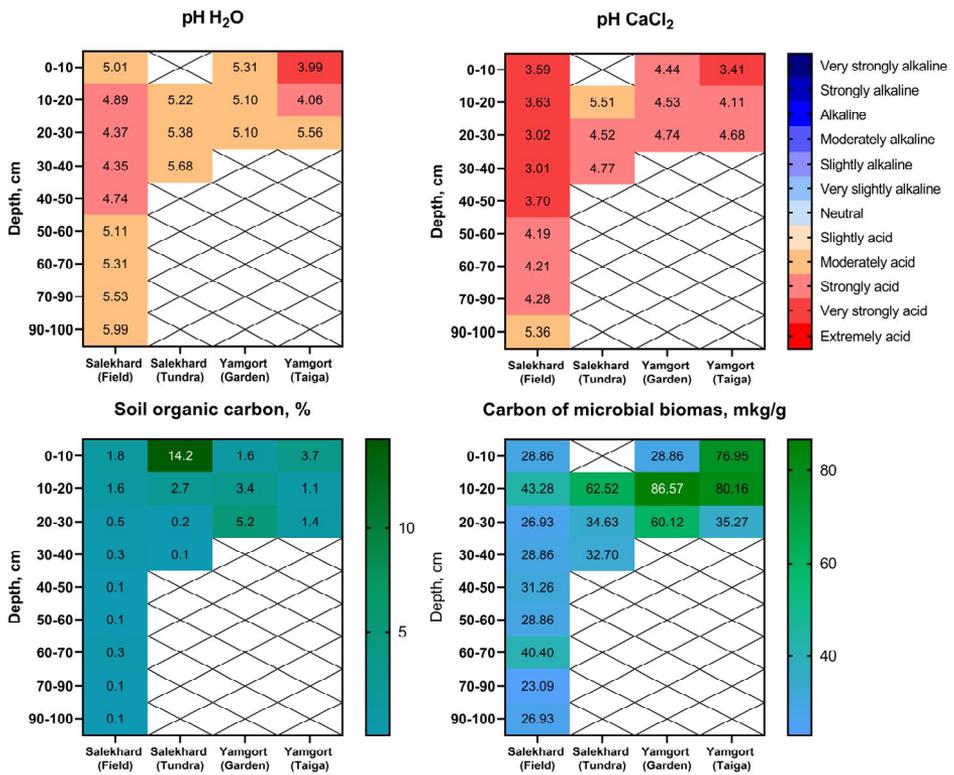


Fig. 3. pH values and carbon content of the studied soils.

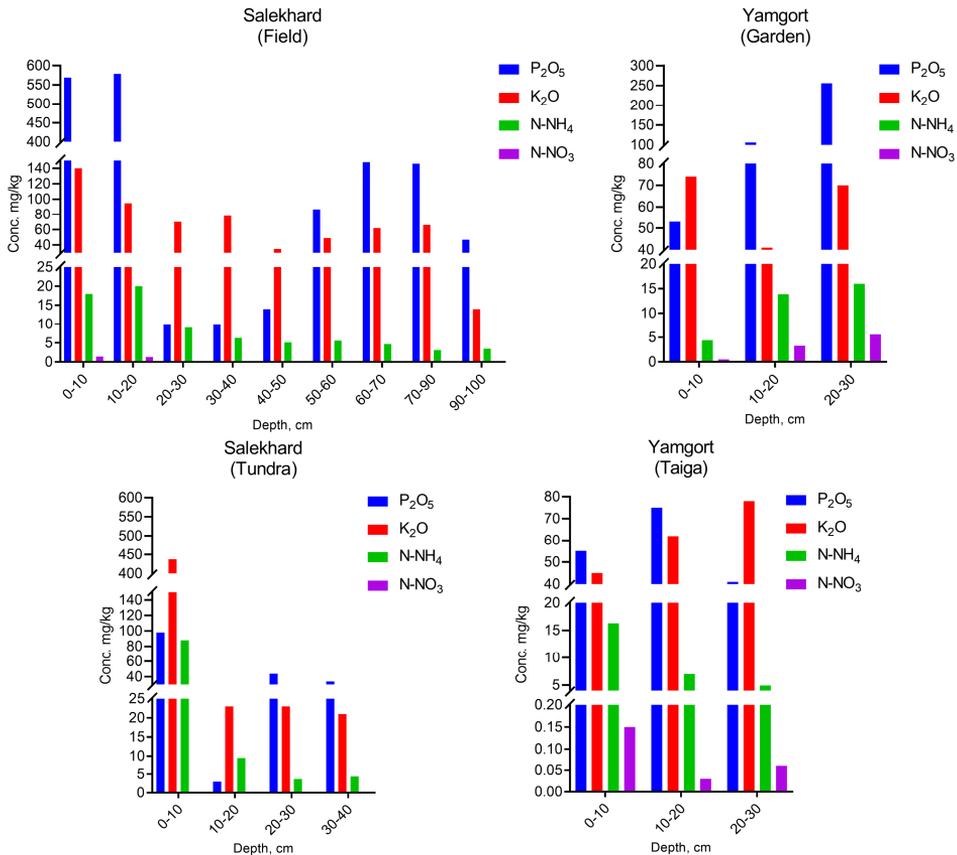
At the same time, the SOC and carbon of microbial biomass in abandoned soils was equal or lower than in pristine soils. The difference in content may be the result of humification and mineralization of or-

ganic matter in the more aerated soils of young deposits (soil becomes looser when plowed and becomes saturated with air) compared to the pristine soils, which contain large amounts of coarse organic mat-

ter of litter and coarse-humus horizons. Separate samples (fivefold repetition for each horizon) of the same horizons were selected for soil microbiome studies.

Fallow soils were characterized by a higher content of available forms of phosphorus and potassium in the upper organomineral (postagrogenic) horizons (Fig. 4).

This indicates a high degree of preservation of fertility parameters of previously cultivated soils during their transition to fallow and further existence for two to three years in the fallow state. The benchmark soils of the cryolithozone were characterized by low content of nutrients.



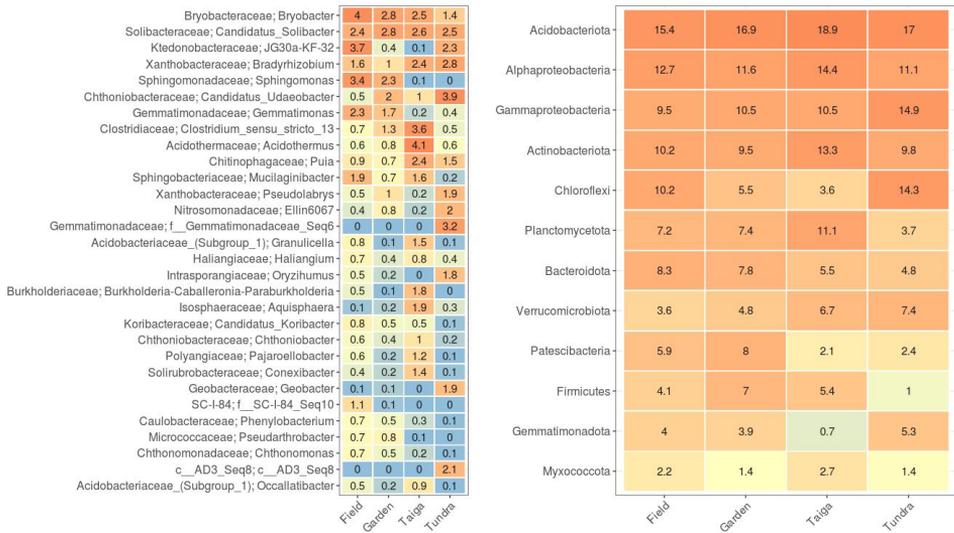
**Fig. 4.** Nutrients content.

### Microbiological research

The results of the microbiological studies are shown in Fig. 5. Sequencing of the 16S rRNA gene libraries was performed for four sampling points: (1) Salekhard city: field of Yamal agro station: two-year

fallow – field, and mature tundra outside the city – tundra, (2) Yamgort village: vegetable garden, one-year fallow – garden, and mature taiga outside the village – taiga.

MICROBIOME OF FORMER AGRICULTURAL CRYOGENIC ECOSYSTEMS



**Fig. 5.** Relative representation (in %) of major phylotypes: individual phylotypes on the left; phyla on the right. Orange stands for the highest and blue – for the lowest values.

Taxonomic analysis of soil microbiomes revealed 33 bacterial and archaeal phyla, among which representatives of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroides*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Patescibacteria*, *Planctomycetes*, *Thaumarchaeota* and *Verrucomicrobia* were dominant. Analysis of alpha- and beta-diversity showed that undisturbed (natural) soils differed significantly (Bray-Curtis distance Permanova test between sites – p-value < 0.001,  $R^2 = 0.56$ ) from anthropogenic-disturbed (including agrogenic) soils in terms of microbial biodiversity indices. Thus, according to the Shannon and Simpson indices, alpha-biodiversity is higher in the soils of fields and orchards. The average values of the Shannon index for the field soil are 6.9, the garden – 7.4, while for mature taiga and tundra 6.6 and 6.3, respectively. A similar picture emerges when calculating the Simpson index. The soils of the urbanized zone of Salekhard differed from all the other studied soils by the number of OTUs and beta biodiversity. At the same time, soils developed in the recreational

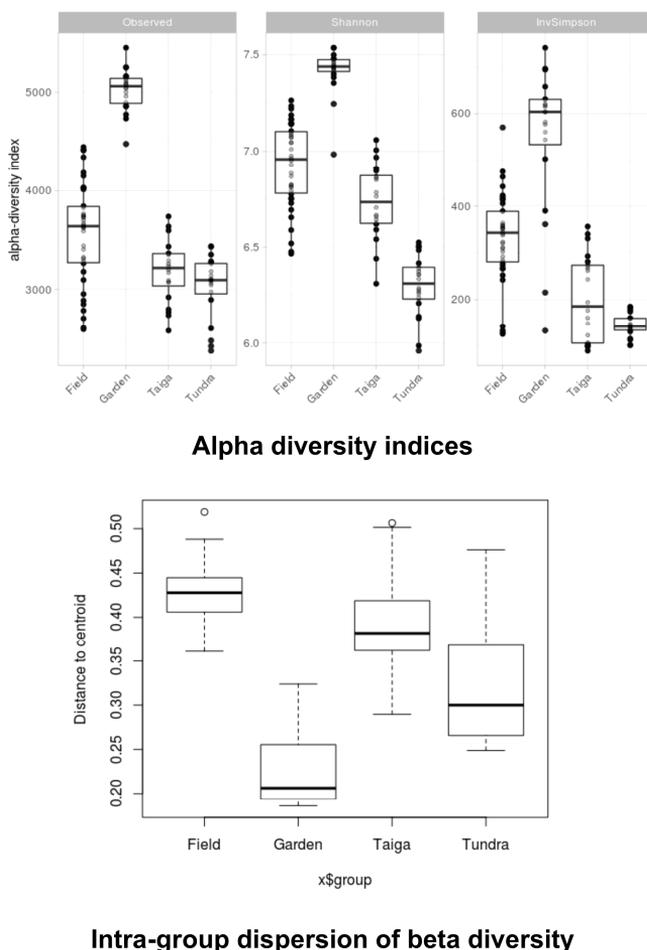
zone of Salekhard demonstrated less pronounced profile differentiation of the microbiome (former agrolandscape fields and former vegetable garden soils). Thirteen of the 30 phyla identified were found to explain more than 99% of the total microbial diversity. This study revealed the most common types of soil microorganisms in the samples collected from naturally and anthropogenically contaminated soils: *Firmicutes* (average 26.86%), *Proteobacteria* (average 23.41%) and *Actinobacteria* (average 15.45%). *Firmicutes* phylum was found to be mainly distributed in soils after arable exposure, which are the most arid (during summer droughts) and more aerated. *Proteobacteria* were mainly described in mature tundra soils, and the presence of *actinobacteria* might be explained by the location of the study area in a relatively humid part of the Yamal Peninsula. The greatest number of observed taxonomic units (OTU) is in anthropogenic soils from former fields, as this area has been intensively used for agriculture during the last decades. At the same time, the lowest number of OTUs was found in

the most technogenically polluted part of Salekhard.

The taxonomic composition (Fig. 5) of the studied soils was generally characteristic of the soils of the cryolithozone. The tundra soils were characterized by an increase in the autotrophic anaerobic component of the microbiome (*Chloroflexi*). Soils of fallow lands were characterized by the absence of archaeal nitrogen-reducing component (*Nitrososphaera*) and by the presence of a high proportion of nitrogen-fixing microorganisms (*Bradyrhizobium*, *Alphaproteobacteria*), which indicates that even for the soils of agrocenoses a significant lack of available forms of nitrogen

in the soil is typical. In addition, in all the studied points there is a high proportion of representatives of *Acitobacteriota* phyla, which are rather characteristic of natural habitats. The proportion of *Actinobacteria* in the studied soils is low compared to the taiga soils of natural and anthropogenic habitats, which is to some extent related to the specificity of these soil habitats.

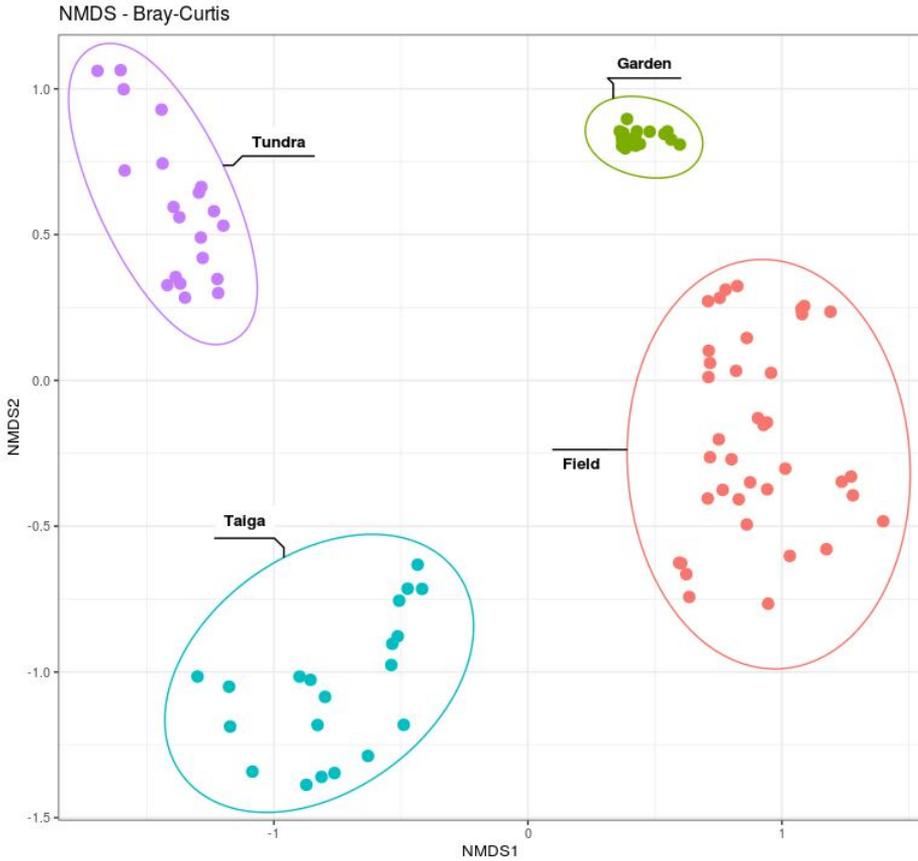
Taxonomic diversity of the studied microbiomes contrasts depending on the sampling point (Fig. 6). At the same time, it should be noted that the intragroup dispersion of beta diversity differs in the microbiomes depending on the studied soils.



**Fig. 6.** Alpha diversity indices and intra-group dispersion of beta diversity.

The alpha-diversity indices (Fig. 7) showed a significant increase for agroecosystems (more for the Yamgort vegetable garden, less for the experimental station) compared to both the taiga and tundra control soils. This may indicate the formation of a greater number of ecological niches in the profiles of agro-soils compared to background soils. Thus, plowing and sub-

sequent use of soils in agriculture may contribute to an increase in microbial biodiversity, but this does not mean that this process is beneficial to yields improving. Just at this stage of research development, this fact should be taken into account, including in terms of possible invasive microbiological risks when advancing agriculture in the northern direction.



**Fig. 7.** Beta diversity plot - NDMS by Bray-Curtis (replicates). All sampling points are contrastingly different from each other.

In turn, it was shown that within-group dispersion of beta diversity was inversely correlated with microbiome richness (Pearson correlation:  $-0.65$ ,  $p$ -value  $< 0.001$ ). Apparently, the adaptation strategies of the

soil microbiome during the formation of agroecosystems in the Arctic may have a different (in this case rather inverse) dynamics compared to the soils of the boreal belt.

## Conclusions

For the first time soil microbiome of mature and abandoned agricultural soils of the central part of Yamal region was investigated with the use of modern methods of soil metagenomics. Data on taxonomy composition of mature soil has been compared with taxonomy diversity of former agrogenic soils. Analysis of the beta diversity of microbial communities showed that the soil of mature tundra has a less differentiated microbiome composition across the soil profile, while soils from anthropogenically affected areas of Salekhard may differ in microbiome composition when comparing the upper and lower soil horizons. The described microbial community in mature soil demonstrates many bacterial taxa common to the Arctic region.

Agricultural development of the Arctic territories leads to radical transformation of the soil cover of the region. As the results of research have shown, initially barely suitable for agriculture lands in the process of their reclamation achieve high quality. There are changes in the acid-alkaline regime, and a high content of basic nutrients is observed. Microbiome diversity also changes significantly upwards.

Usually more southern soils under the agricultural impact the diversity of soil ecological niches decreases due to homogeni-

zation of soil profile. In the case of southern soils, we observe processes of soil degradation under agricultural impact. In contrast, in northern soils investigated, the increase of soil biodiversity in case of arable soils was detected. In fact, these are processes of soil cover evolution, agricultural use leads to the generation of new types of soils in the Arctic regions, which are not originally found there. High concentrations of nutrients have been found in abandoned soils, making them potentially suitable for rewetting in agriculture. In addition, the involvement of abandoned soils in agriculture will help to prevent the invasion of atypical microbiota in polar ecosystems. The increase of the microbiome diversity may be not linked directly to the soil fertility, but can indicate the possibility of its growth.

Investigations focused on the processes of post-agrogenic transformation of soils in the Arctic regions should be continued or even transferred to a monitoring level. Assessment of the rate of transformation of these soils, identifying the frequency of changes in the microbiome diversity, will help in further planning of agricultural development of the North, Yamal Peninsula in particular.

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