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Microbial Communities of Peaty Permafrost Tundra Soils along the Gradient of Environmental Conditions and Anthropogenic Disturbance in Pechora River Delta in the Eastern European Arctic

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Abstract: Microbial communities play crucial roles in the global carbon cycle, particularly in peatland and tundra ecosystems experiencing climate change. The latest IPCC assessments highlight the anthropogenic changes in the Arctic peatlands and their consequences due to global climate change. These disturbances could trigger permafrost degradation and intensification of the biogeochemical processes resulting in greenhouse gas formation. In this study, we describe the variation in diversity and composition of soil microbial communities from shallow peat tundra sites with different anthropogenic loads and applied restoration interventions in the landscape of remnant fragments of terraces in the Pechora River delta, the Russian Arctic, Nenets Autonomous Okrug. The molecular approaches, including quantitative real-time PCR and high-throughput Illumina sequencing of 16S RNA and ITS, were applied to examine the bacterial and fungal communities in the soil samples. Anthropogenic disturbance leads to a significant decrease in the representation of Acidobacteria and Verrucomicrobia, while the proportion and diversity of Proteobacteria increase. Fungal communities in undisturbed sites may be characterized as monodominant, and anthropogenic impact increases the fungal diversity. Only the verrucomicrobial methanotrophs Methyloacifiphilaceae were found in the undisturbed sites, but proteobacterial methanotrophs *Methylobacterium-Methylorubrum*, as well as different methylotrophs affiliated with Methylophilaceae, and Beijerinckiaceae (*Methylorosula*), were detected in disturbed sites.

Keywords: arctic ecosystems; disturbed peatlands; bacteria; fungi; microbial diversity; qPCR; Illumina sequencing

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1. Introduction

Climate warming is becoming increasingly critical, most notably at high latitudes [1]. Anthropogenic impacts exacerbate climate change, increase the vulnerability of ecosystems, and have complex implications for various aspects of human life [2]. Peatlands have a special place in climate change mitigation and adaptation problem solutions [3], which lead on land in terms of carbon stocks, outstrip all other ecosystems in terms of carbon stocks per unit area, influence greenhouse gas fluxes [4], and are carriers of specific biodiversity [5,6].

The conditions in the north (excess of precipitation over evaporation, presence of permafrost) promote peatland formation. The short vegetation period that is compensated by extended daylight provides sufficient ecosystem productivity. Plant remains that have

not fully decayed contribute to peat formation. Thermokarst, frost heaving, and frost cracking form the morphological diversity of peatlands and shallow peat landscapes in the Arctic [7]. The presence of permafrost and, hence, insignificant summer thawing of the soil limits the biogeochemical processes to the upper soil layers, so, in the Arctic conditions, shallow peatlands are functionally close to oligotrophic mires [8]. Even though the Conservation of Arctic Flora and Fauna working group classifies watershed peatlands, including shallow peatlands, under the tundra category, peatlands occupy 12.3%, and, together with shallow peatlands (peat less than 30 cm), 34.8% of the Russian Arctic [9]. They carry critical ecosystem functions, such as greenhouse gas fluxes regulation, protection of the permafrost from thawing, maintenance of the water balance, and diversity of the biota. At the same time, due to the small peat depth, they are the least resistant to anthropogenic mechanical impacts [3]. Disturbance or loss of vegetation cover can quickly lead to peat soil destruction, water, and wind erosion and further peat decomposition. Shallow tundra peatlands in the Arctic are underestimated in terms of their extent, ecosystem functions, services, and robustness against climate change and human impacts [8]. Shallow tundra peatlands in the Arctic are underestimated in terms of their extent, ecosystem functions, services, and robustness against climate change and human impacts [8]. That is one of the reasons that studies insufficiently cover their structural and functional characteristics.

Soil microbiomes play an essential role in soil biochemical processes [10]. Microorganisms are responsible for both the decomposition and formation of organic matter, as well as ensuring the cycling of nutrients in the soil. These processes are crucial for the regeneration of disturbed ecosystems of the Arctic [11]. The composition of microbial communities is decisive for ecosystems' carbon balance and greenhouse gas (GHG) exchange in the rapidly changing environment [12]. Hence, the microbial composition could be an informative indicator for assessing ecosystem disturbances and regeneration status in relation to the key ecosystem functions.

The connections between vegetation, hydrology, soil properties, and microbial communities have been examined in a number of studies carried out in the boreal [13,14] and frozen Arctic peatlands [15–17], while, to date, there is practically no research on the interconnections between biophysical features of ecosystems and microbial community structure in the natural shallow peat tundra and in disturbed landscapes in the Arctic zone.

For practical needs of ecological indication, it would be helpful to designate whether the changes in microbial communities' diversity characteristics follow the biophysical attributes in the course of regeneration changes. The latest publications prove that modern molecular approaches allow for capturing less abundant and uncultured microbial taxa [18,19].

We hypothesized that anthropogenic disturbances of the shallow tundra peatland would modulate the composition of the soil resident microbiome and lead to different patterns of bacterial community shifts according to the gradient of environmental conditions and anthropogenic disturbance. Therefore, the present study aimed to determine if the structure of soil microbial communities reflects the level of disturbance/regeneration in the transformed shallow peatland ecosystems in the Arctic and evaluate their indication capacity.

2. Materials and Methods

2.1. Study Region

The research object is located in the Nenets Autonomous Okrug in the delta of the Pechora River, Barents Sea basin, on the territory of Nenets State Nature Reserve (Figure 1). The climate is a moderately cold Arctic climate. The average annual temperature is −2.4 °C, with an average maximum of 18.9 °C and absolute maximum of 33.9 °C in July and an average minimum of −21.1 °C and an absolute minimum of −47.9 °C in January. The mean annual precipitation is 516 mm and ranges from a minimum of 257 mm to a maximum of 706 mm.

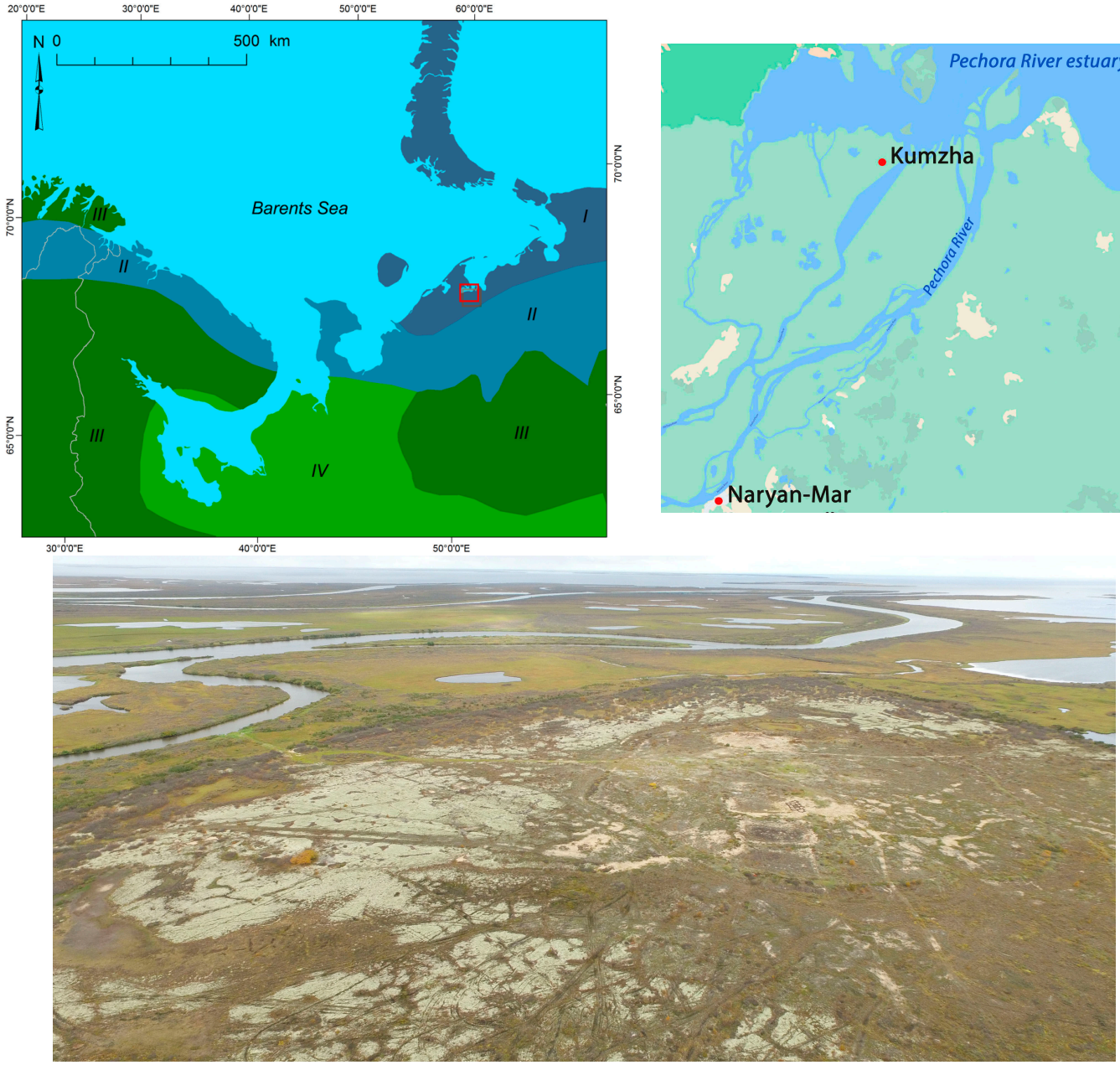

Figure 1. Location of study area in relation to the European mire regions marked with the red frame, the Pechora River delta, and a general view of the site from the quadcopter. The European mire regions are given according to [20], as simplified from [21]: I—Arctic seepage and polygon mire region, II—palsa mire region, III—northern fen region, IV—typical raised bog region.

The study area is located in the southern tundra belt and from the point of mire zoning in the Arctic seepage and polygon mire region [20]. This mire region is characterized by the presence of frozen polygon mires in watersheds and patches of lowland fens in river valleys, as well as other watercourses where permafrost is deep or absent. Occasionally, isolated patches of palsa mires, often degraded, appear on the slopes of river valleys and closer to the coast, where drainage is better [22]. Frozen polygons are usually covered by shallow peat; morphologically and by vegetation, it is often challenging to distinguish polygon mires from polygon shallow peat tundra. The shallow peat tundra covers most of the transgression terraces and lasts up to 50 km from the Barents coast towards the continent. It is found along the coast and in the river deltas on the residual fragments of the terraces. The region's peatlands are significantly less disturbed by human activities than

European peatlands, and the degree of degradation is about 1% [22]. At the same time, the development of infrastructure and transport in this region leads to violations of vulnerable Arctic ecosystems, which is especially dangerous under current climate conditions [23]. The presence of sandy bedrocks in the coastal transgression landscapes and deltas makes the landscape degradation almost irreversible. Shallow peatlands get replaced by sandy dunes that carry different ecosystem functions and services [8].

2.2. Study Sites and Objects

The site under study, the Kumzha site (68°11′ N 53°47′ E, CALM R24A-2), is the part of the slightly elevated (4–10 m asl) remains of the young first inland alluvial terrace (I am) that used to be covered with the relic inland southern tundra vegetation and shallow peat cover. The bedrock is composed of thawed and frozen sand with clay and peat inclusions. An example of the intact peatlands and shallow peat tundra on the residual terrace can be found on Kashin Island (68°14′ N 53°51′ E, CALM R24A—[19]), which is also a remnant of the first terrace and maintained in its natural status by the Nenets Nature Reserve [20].

The terraces' fragments cover not more than 5% of the delta area and are valuable habitats under solid anthropogenic pressure, given that they are handy places used for the development of all types of infrastructure, including traditional use and recreation (hunting and fishing facilities), and use by the oil and gas industry. Specifically, the Kumzha site was used as a natural platform for condensed gas exploration in the late eighties of the 20th century.

The exploration installations included two rows of ridges bounding the drilling pad, depressions from which the ground for artificial ridges was taken, two drilling wells, underground sludge pits for drilling waste water storage, plots cleared for camps and storage facilities, unpaved roads from local ground, and decking roads (Figure 1).

Since the end of the exploration works, the area has been under natural regeneration and interrupted by several interventions for rehabilitation. In 2008, the technical stage of site rehabilitation aimed at removal of metal trash brought additional disturbances to the ecosystem. In 2014, several experimental sites for ecosystem restoration were set up. In 2016, the drilling pad wellheads were removed, and routine rehabilitation was implemented, such as ploughing and grass sawing. The site was the subject of detailed study in the framework of the Circumpolar Active Layer Monitoring (CALM) program [24] and ecological restoration experiments supported by the UNDP program [23,25].

The typical initial landscape structure of the study site was retrieved from the undisturbed area, e.g., on the Kashin Island. The concept of peatland structure diversity was applied to identify and describe initial spatial landscape units at the level of micro-landscapes [12]. Further, the disturbed and relevant reference micro-landscapes were paired by predicted successional connections.

The following three classes of micro-landscapes were identified at the natural site: flattened areas parts on the top of the island and flat terraces of the slope are covered by ombrotrophic mires or bogs with peat depth of more than 50 cm (half a meter) and the dense cover of sphagnum mosses, lichens, small sedges, and dwarf shrubs; the drained gentle slopes covered with shallow (less than half a meter) peat and lichen moss dwarf shrub tundra; and the poor minerotrophic fen with mesotrophic mire vegetation, including brown mosses, tall sedges, and willows.

The area under study presents a diverse mosaic of patches with different disturbance character and intensity distributed through three main initial landscape types. The following types of disturbances we used for designation of the testing sites: the areal disturbances, that are the most heavy and where both vegetation and peat cover are destroyed at an area rate of more than the first dozens of square meters; the linear disturbances that include all types of roads, ditches, and artificial ridges where vegetation and peat cover are destroyed at the limited locations along the artificial linear structures; and the scattered disturbances that include the areas with sporadic distribution of patches where vegetation and soil are disturbed at the limited space, and self-regeneration is still visible.

The study design was aimed to cover the diversity of habitats that are formed after the transformation of each of three initial micro-landscapes by each of three types of disturbances and reference sites. For lowland micro-landscapes, the ecological restoration was applied. The newly created habitats were also included in the research. Not all combinations were available for study. Eleven sampling plots were chosen to reflect the diversity of the habitats (Table 1).

Table 1. General characteristic of the sampling sites.

Site ID	Coordinates	Elevation, m	Initial Micro-Landscape	Dominating Current Vegetation	Disturbance Type	Restoration Action
M30	N 68°11′31.25″; E 53°46′41.29″	4.0	Ombrotrophic mires of flat landscapes	Sedge, lichen, sphagnum hummocks within fen	Reference	No
M24	N 68°11′36.51″; E 53°46′46.35″	6.1	Shallow peat tundra on the slope	Lichen, dwarf shrubs with dwarf birch	Reference	No
M27	N 68°11′38.58″; E 53°46′47.18″	4.0	Fens on the lowlands and riverine habitats	Brown moss forbs, willow dwarf birch	Reference	No
M19	N 68°11′35.70″; E 53°47′11.92″	6.5	Ombrotrophic mires of flat landscapes	Grass, moss mesophilic with shrubs	Areal	Self-regeneration
M3	N 68°11′37.78″; E 53°47′15.74″	6.1	Shallow peat tundra on the slope	Grass, forbs xerophilic with crust	Areal	Self-regeneration
M8	N 68°11′37.04″; E 53°47′15.68″	5.4	Fens on the lowlands and riverine habitats	Grass, forbs xerophilic with crust	Areal	Self-regeneration
M9	N 68°11′38.27″; E 53°47′15.68″	5.4	Fens on the lowlands and riverine habitats	Grass, forbs xerophilic with lichens	Areal	Ecological restoration
M39	N 68°11′35.55″; E 53°46′50.42″	5.6	Ombrotrophic mires of flat landscapes	Lichen, dwarf birch	Scattered	Self-regeneration
M29	N 68°11′35.81″; E 53°46′50.50″	5.6	Shallow peat tundra on the slope	Lichen, dwarf shrubs	Scattered	Self-regeneration
M31	N 68°11′37.49″; E 53°46′54.36″	5.2	Shallow peat tundra on the slope	Moss, forbs, willow dwarf birch	Linear	Self-regeneration
M4	N 68°11′38.94″; E 53°46′47.45″	4.0	Fens on the lowlands and riverine habitats	Brown moss, forbs with willow	Linear	Self-regeneration

The selected plots represented, at the time of sampling (2020), different types of habitats representing stages of vegetation regeneration succession with a wide range of plant communities (Figure 2). The vegetation cover of the plots was described by common geobotanical methods, and all vegetation species were classified according to the stratification of the plants [26]. The spatial structure of the vegetation cover was established using the methods of large-scale geobotanical mapping. The vascular plant, moss, and lichen species were preliminarily identified in the field, sampled, and confirmed by the specialists based on the herbarium; the samples are available for verification.

2.3. Sample Collection and Soil Characterization

Sampling was done in August 2020 at the selected sites. The "envelope" inclusive sampling design was applied. The vegetation relevé was bounded by a circle with the diameter of five meters. Triplicate soil samples were taken by cylinder from the depth 0–4 cm; samples were collected by the sampler at 5 × 5 cm in every corner of every of five 50 × 50 cm squares and further mixed. The vegetation relevé included a list of presented vascular species, bryophytes, and lichens with their phenological status, height, and cover, as well as cover and height of every plant community layer. The active layer depth (distance to permafrost roof) was measured by the probe corer at 1.80 m length. In some cases (sites M9, M39, M30, and M24) the deep coring data were available due to the presence of CALM plot. The soil subsamples were homogenized and stored in the cooling incubator (2–5 °C) before being transferred to the laboratory. Samples for DNA extraction were immediately frozen.

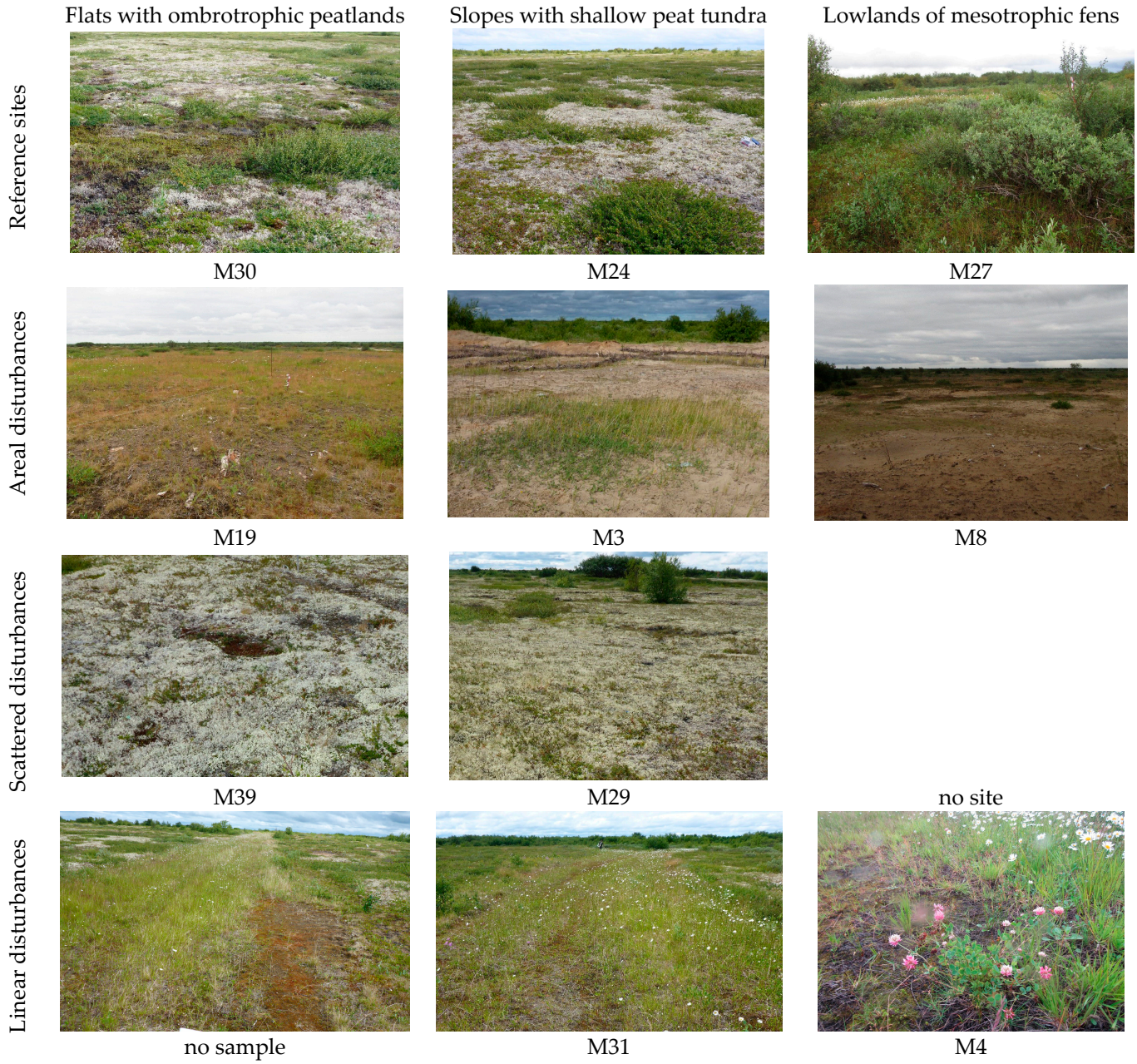

Figure 2. Characteristic types of micro-landscapes represented in the studied peatlands. The numbers indicate the monitoring plots.

The main soil parameters were determined using standard procedures. Values of pH in soil were measured using a pH150 m (1:5 soil: H_2O ratio). Bulk density was determined by the soil core method. The dry matter content of the soil was determined by drying the samples (105 °C, 12 h), and OM content was analyzed by loss upon ignition (475 °C, 4 h). Carbon and nitrogen content were measured using a Vario Max element analyzer (Elementar Analysensystem GmbH, Langenselbold, Germany).

2.4. DNA Extraction, qPCR Assays, and Sequencing

We used the Power Soil DNA Isolation Kit (Qiagen, Carlsbad, CA, USA) to extract total soil DNA from 0.25 g of rhizosphere soil samples, following the manufacturer's protocol. To evaluate the DNA concentration and purity, a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used.

The total abundance of bacterial (16S rRNA genes) and fungal (18S rRNA genes) communities was evaluated by quantitative PCR assays (qPCR) with the primers [27] for bacteria and [28] for fungi. Briefly, the synthesis of the first cDNA chain from a single-stranded RNA matrix was carried out using MMLV reverse transcriptase (revertase) according to the manufacturer's recommendations (CJSC Eurogen, Moscow, Russia). The qPCR reactions were carried out in real-time in a PCR buffer-RV (Syntol LLC, Moscow, Russia), in the presence of SYBR Green I and a passive reference dye ROX using the CFX96 Touch Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The standard curves were prepared using *Escherichia coli* for bacteria and *Penicillium chrysogenum* for fungi. Each qPCR mix consisted of 4.2 µL sterilized water, 10 µL SYBR green master mix, 0.4 µL of each primer (0.4 pmoles/ µL) and 5 µL of diluted template DNA for a final reaction volume of 20 µL. The PCR program included a polymerase activation stage for 5 min at 95 °C, and the next 40 cycles were 15 s at 95 °C, 45 s at 62 °C.

High throughput sequencing was applied to evaluate microbial diversity. The variable V4 region of the bacterial 16S rRNA gene was amplified using universal primers 515F and 806R (https://doi.org/10.17504/protocols.io.nuudeww) (accessed on 26 November 2021), and the fungal ITS1 region was amplified with primers ITS5 and 5.8S_fungi [29], together with linkers and unique barcodes. PCR was performed on a T100 Thermal Cycler device (Bio-Rad Laboratories, Hercules, CA, USA) in 15 µL containing 0.5 units of Q5® High-Fidelity DNA Polymerase (New England BioLabs Inc., Ipswich, MA, USA), 1X Q5 reaction buffer, 5 pmol of each of the primers, 3.5 mM dNTP (CJSC Eurogen, Moscow, Russia) and 1–10 ng of the DNA matrix. The PCR program included a denaturation stage at 94 °C for1 min, amplification for 35 cycles (94 °C—30 s, 50 °C—30 s, 72 °C—30 s), and final elongation at 72 °C for3 min.

Further analysis was performed following the Illumina protocol (16S Metagenomic Sequencing Library Preparation) on the Illumina MiSeq instrument (Illumina, Inc., Foster City, CA, USA) using the MiSeq Reagent Kit v3 (600 cycles) (Illumina, San Diego, CA, USA) running 2 × 300 bp paired-end reads.

2.5. Sequence Data Processing and Statistical Analysis

Pre-processing of the Illumina sequencing reads included removal of the adapters and indices using the Cutadapt ver. 1.11 [30], as well as denoising, combining paired reads, and deleting chimeras using the Dada2 package implemented in the QIIME2 package, ver. 2019.7 [31].

After filtering, high-quality sequences were obtained and included for subsequent analyses. The OTU abundance of each sample was standardized by using the lowest level of sequence depth as a reference. The OTUs were assigned de novo at 97% identity level. The taxonomic classification of the obtained amplicon sequence variants was also performed using SILVA v.138 database containing data for the SSU rRNA genes [32]. Features with an abundance of less than 10 or that presented in only one sample were filtered out to remove the possible PCR artifacts or chimeras. The OTUs were assigned de novo at 97% identity level. The taxonomic classification of the obtained amplicon sequence variants was also performed using SILVA database containing data for the SSU rRNA genes [32].

Further processing, including the construction of a phylogenetic tree using the Fast-Tree algorithm [33], for the calculation of α and β diversity, was performed within the QIIME2 package [31] and the plugins implemented in it. The diversity indices reflecting the predicted species richness (Faith's PD) and the degree of evenness (Pielou's index) were considered to assess the α- diversity.

All measurements were performed in at least three repetitions. For each sample, the mean and standard deviation were calculated in the Excel software product (Microsoft Corp.). Statistical processing was carried out using the Statistica 8.0 program (StatSoft Inc., Tulsa, OK, USA). For paired comparisons, the Student's criterion (*t*-test) was used, and the criteria were considered statistically significant at $p < 0.05$.

3. Results

3.1. The Characteristics of the Sampling Sites and Soil Physicochemical Properties

Characteristics of the vegetation cover and soil profile of the sampling sites are presented in Table 2. Reference sites (M24, 27, and 30) were characterized by 100% undisturbed peat soils with a peat layer of 10–25 cm. They were almost 90% covered with vegetation, including mosses, lichens, and grasses. In the case of peatland micro-landscapes (M30) and mesotrophic peatland micro-landscapes (M27) presented shrub layers. In disturbed sites, the peat layer, if still present in a fraction of the area, was no more than 1–3 cm thick. There was no difference in thawing depth and thickness of the active layer between undisturbed and disturbed areas. The exception was the ombrotrophic peatland micro-landscape, where the permafrost thawing depth was only 1.25 m deep. In all other cases, the thawing depth of about 2 m was not detectable by gauging and was retrieved from the CALM plot data [25]. At the same time, all disturbed areas differed from the reference areas in terms of the depth of soil water table levels. For undisturbed sites, the water table was at a depth of 3–7 cm; on disturbed sites, it was at a depth of more than 30 cm. The exception was the M4 site, where the water table was at a depth of 15 cm.

A summary of the basic soil parameters for the studied soils is presented in Table 3. The key soil physicochemical parameters revealed correlations with environmental conditions and anthropogenic disturbance. The pH values were predominantly slightly acidic. The pH was between 4.55 and 5.37 (Table 4). The exception was point M19, where the pH was 6.25, which seems to be related to anthropogenic contamination. This can be indirectly indicated by the sulfur content, which was not observed at the other sites. The lowest values were found in the samples containing peat and moss material and the highest in the samples of turf with sand. Soil bulk density showed a generally increasing trend with depth of disturbance (M3, M8, M9) across the landscape types, with large variabilities at a given landscape type. The bulk density of peat soil on undisturbed plots was 0.49–0.72 g/cm^3, while on disturbed plots, in most cases, it was well over 1 g/cm^3, due to high mineral particle content. Ash content on undisturbed plots was 64–77%, while on disturbed plots, in most cases, it approached or even exceeded 90%. The organic content approached or exceeded 30% only for undisturbed plots. For disturbed plots, this value was observed only for site M39; for the rest, it was the first percent.

Unlike the bulk densities, the soil OM content decreased in the disturbed site, and the highest content was found in undisturbed reference sites M24, M30, M27, and slightly sparse disturbance site M39. Soil total C (TC) and N (TN) ranged from 1.4 to 23.4% and from 0.05 to 0.85%, respectively. Organic carbon content was highest (up to 35.8%) in M30, which was the ombrotrophic mire reference site. The contents of nitrogen varied from 0.05 to 0.85%. In addition to the OM content, the soil C: N ratio can provide additional insights on the characteristics of the soil carbon and its potential impact on carbon decomposition. The C:N ratio has been suggested to be a better indicator of the degree of OM decomposition when compared to soil carbon or nitrogen content alone [34]. The analysis of C: N mean values revealed that the soils of the Kumzha site had the widest ratio, likely as a result of the gradual distribution within the illuvial profile. The wide C: N ratio (from 19 to 65) suggested a deficit of nitrogen, inhibited decomposition of the plant remnants, and accumulation of the organic material. This observation supports data reported previously for permafrost-affected soils in various sectors of the Arctic [35] and is needed to properly investigate soil carbon and nitrogen pools in studied sites.

Table 2. Descriptive characteristics of soil profile and vegetation cover of the sampling sites.

Site ID	Organic Soil Layer			Underlying Bedrock	Vegetation Cover, %				Litter Cover, %	Number of High Plants Species	Average Vegetation Height, cm		Soil Water Level, cm	Active Layer Depth, m
	Type	Cover, %	Depth, cm		Overall	Shrub	Herb	Moss, Lichen			Shrub	Herb		
					Reference sites									
M30	peat	100	12	sandy loess	95	40	50	90	15	19	60	25	−3	1.25
M24	peat and litter	100	10	sandy loess	90	3	40	85	0	23	100	40	−5	2.2
M27	moss, litter, turf, peat	100	25	sand	90	30	70	30	0	15	120	40	−7	>1.80
					Areal disturbance									
M19	turf, moss, litter	80	3	sand and technogenic loam	90	5	70	20	20	19	15	35	>30	>1.80
M3	litter, crust, and lichen	50	2	sand	30	0	20	10	10	6	0	30	>30	>1.80
M8	crust and lichen	30	2	sand	35	0	30	30	5	11	0	30	>30	>1.80
M9	crust	5	1	sand	20	0	20	5	5	11	0	30	>30	2.2
					Scattered disturbance									
M39	peat and litter	100	6	sand	100	40	60	50	20	14	80	25	>30	2
M29	moss, lichen, turf, peat	80	6	sand	90	0	75	60	0	13	0	40	>30	>1.80
					Linear disturbance									
M31	turf and peat	90	6	sand	70	0	70	20	20	11	0	30	>30	>1.80
M4	litter and crust	65	2	sand	90	0	80	60	10	14	0	15	−15	>1.80

Table 3. Physicochemical parameters of the topsoil material in the studied sites.

Site ID	Material	pH	Bulk Density, g/cm^3	Moisture Content, %	Ash Content, %	Organic Matter, %	TC, %	TN, %	C:N	TS, %
Reference sites										
M30	peat and moss	4.94	0.54	64.00	64.2	35.8	n.d.	n.d.	n.d.	n.d.
M24	peat, moss, lichen	4.55	0.72	38.00	72.3	27.7	n.d.	n.d.	n.d.	n.d.
M27	moss, turf and peat with sand	5.03	0.49	54.00	76.8	23.16	16.2	0.85	19	0.00
Areal disturbance										
M19	turf with sand	6.25	1.15	12.90	89.5	10.55	5.2	0.08	65	1.07
M3	sand and humified sand	5.37	1.35	10.38	98.1	1.88	7.4	0.23	32	0.00
M8	sand and humified sand	5.30	1.34	5.70	98.9	1.09	1.4	0.05	28	0.00
M9	sand and humified sand	5.34	1.44	10.44	99.1	0.91	1.4	0.05	28	0.00
Scattered disturbance										
M39	lichen and peat	5.32	0.26	39.00	68.87	31.13	23.4	0.61	38	0.00
M29	moss, turf with sand	4.63	0.94	12.80	93.8	6.23	6.5	0.24	27	0.00
Linear disturbance										
M31	litter and turf with sand	5.32	1.26	6.70	98.8	1.20	2.2	0.07	31	0.00
M4	moss, crust and turf with sand	4.90	1.01	16.60	96.4	3.56	4.3	0.19	23	0.00

n.d.—not determined.

Table 4. The abundance of bacteria and fungi, as indicated by the number of 16S rRNA and ITS copies measured using quantitative PCR (qPCR).

Sample ID	Bacteria, 16S rRNA Copies g^{-1}	Fungi, ITS Copies g^{-1}
Reference sites		
M30	$10.50 \pm 2.1 \times 10^9$	$14.56 \pm 4.2 \times 10^6$
M24	$1.30 \pm 0.8 \times 10^{10}$	$7.20 \pm 2.2 \times 10^7$
M27	$8.32 \pm 1.8 \times 10^9$	$6.94 \pm 2.2 \times 10^7$
Areal disturbance		
M19	$3.22 \pm 1.2 \times 10^{10}$	$4.42 \pm 0.4 \times 10^7$
M3	$2.42 \pm 0.8 \times 10^9$	$15.72 \pm 4.4 \times 10^5$
M8	$4.98 \pm 0.8 \times 10^9$	$7.98 \pm 2.4 \times 10^6$
Scattered disturbance		
M39	$3.24 \pm 0.8 \times 10^{10}$	$5.52 \pm 1.1 \times 10^6$
M29	$9.46 \pm 2.2 \times 10^9$	$14.52 \pm 5.4 \times 10^6$
M9	$6.20 \pm 2.3 \times 10^9$	$2.14 \pm 0.6 \times 10^7$
Linear disturbance		
M31	$4.36 \pm 1.1 \times 10^{10}$	$5.72 \pm 1.7 \times 10^7$
M4	$2.78 \pm 0.6 \times 10^{10}$	$2.70 \pm 0.3 \times 10^7$

3.2. Results of qPCR Estimates of Bacterial and Fungal Abundance

Quantitative PCR (qPCR) was applied to determine the abundance of bacteria and fungi across disturbance gradients. Strong decreasing trends were observed from low to high disturbance levels (Table 4). The number of bacteria genes copies increased nearly tenfold, and the numbers of fungal copies changed to a lesser extent.

3.3. Taxonomic Diversity and Microbial Community's Composition

The number of the prokaryotic 16S rRNA gene reads decreased in samples of the disturbed sites M3, M8, and M9 (Figure 3a), especially in the shallow peat tundra on the slopes. The largest number of readings was found in the rich and transition fens reference site M27. This correlated with the values of the number of prokaryotes obtained for these objects by quantitative PCR.

In the soil of undisturbed sites, at the phylum level, the bacterial community was sufficiently aligned, and the dominant phyla were Proteobacteria (30–37%), Acidobacteria (33–45%), Bacteroidetes (10–13%), and Verrucomicrobia (9–12%) (Figure 3b). Representatives of other phyla made up no more than 10–15% of the total number of prokaryotes. Anthropogenic impact leads to changes in the structure of communities at the phylum level. There was a significant decrease in the proportion of Acidobacteria (15–8%) and Verrucomicrobia (4–2%), but the proportion of Proteobacteria increased (45–49%). There was a significant increase in the relative abundance of representatives of the phylum of prokaryotes, whose share in the communities of undisturbed tundra soils was less than 1%, and these included Actinobacteria (3–37%) and Firmicutes (5–8%). For representatives of Planctomycetes, Cyanobacteria, Bacteroidetes, Gemmatimonadetes, Crenarchaeota, and candidates of AD3 and WPS-2 groups, the differences were statistically unreliable.

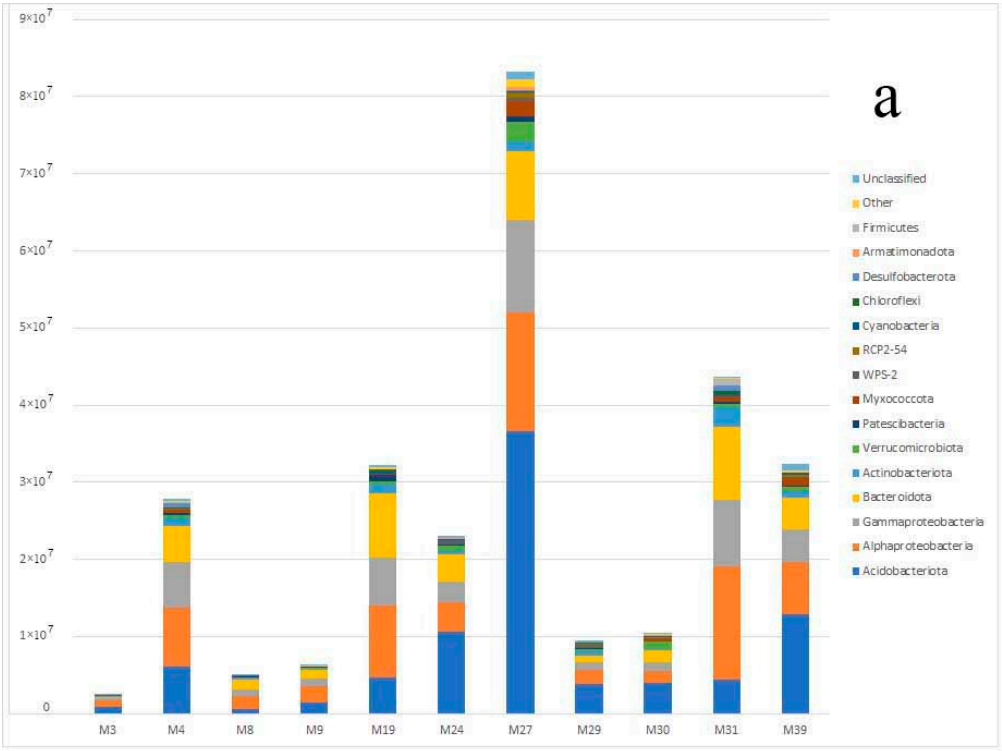

Figure 3. *Cont.*

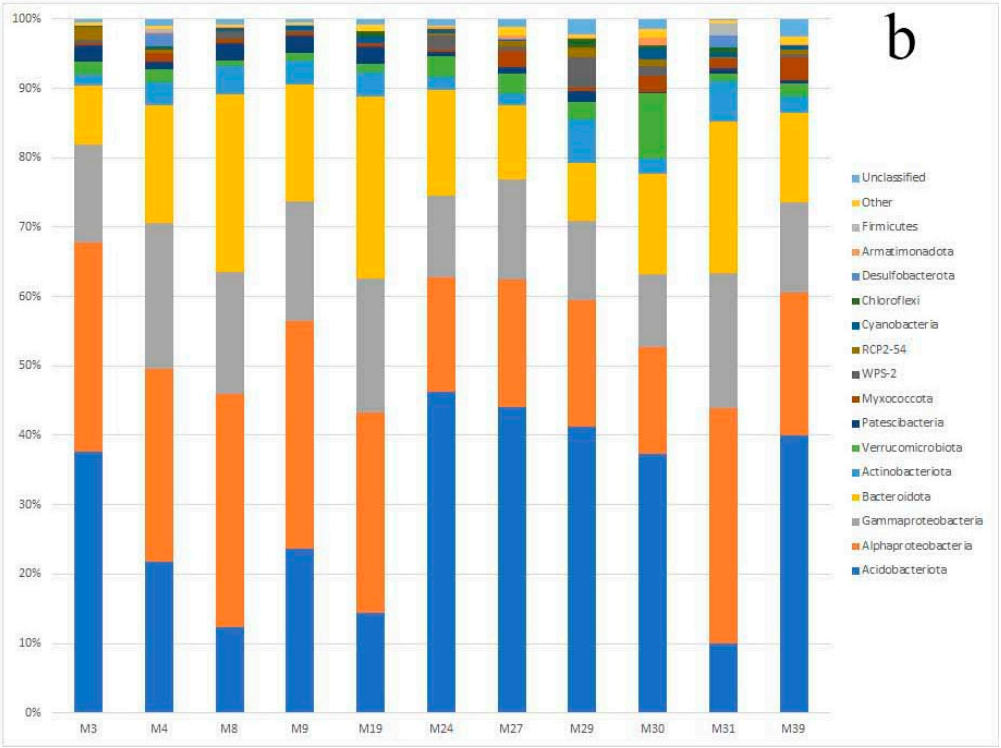

Figure 3. The number of bacterial sequences (**a**) and the taxonomic structure at the phylum level of the bacterial community of the studied soils in % of the total number of sequences in the sample (**b**) according to high-throughput sequencing of 16s rRNA. The median value of the three replicate samples per sampling site is presented. The taxa constituting >1% in at least one library are listed.

Analysis of the microscopic fungi in the undisturbed sites showed a significant presence of Ascomycota (30–83%) and Basidiomycota (9–31%). In the samples of disturbed sites, an increase in taxonomic diversity was observed due to representatives of the phyla Mucoromycota, Glomeromycota, and Cilophora (Figure 4). A significant part of the obtained sequences referred to uncultivated organisms.

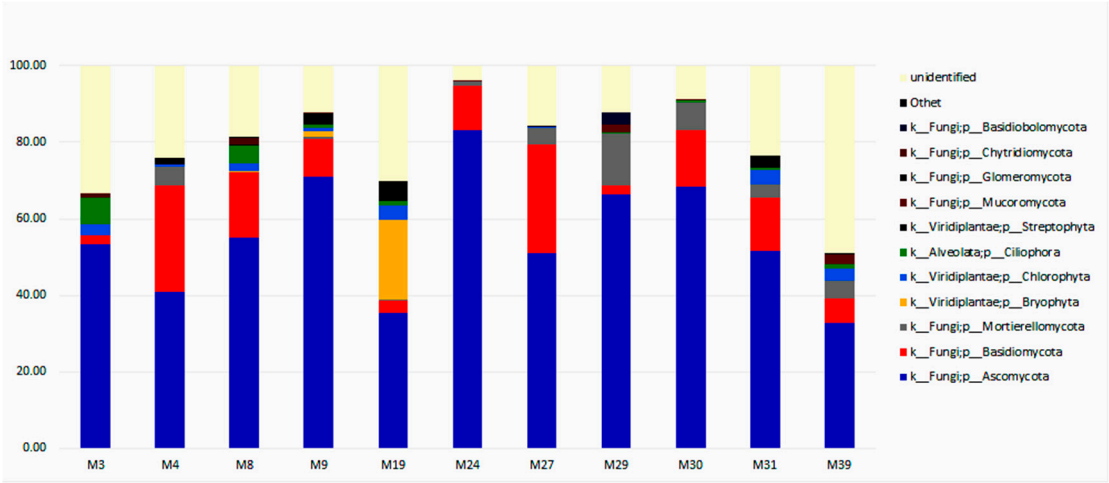

Figure 4. Taxonomic structure of the fungal community at the phylum level in the studied soils, % of the total number of sequences in the sample according to ITS data.

To explore bacterial and fungal species richness of a particular sample, we calculated phylogenetic diversity using the Faith's PD index, and species evenness using Pielou's

index and Shannon's index (Table 5). The assessment of the α diversity of the bacterial community revealed significant differences in the variants. An increase in the overall diversity, the number of OTU, in disturbed sites was noted. This was confirmed by the values of the Chao and Shannon indices.

Table 5. Alpha diversity indices of the soil bacterial communities based on the 16S rRNA gene analysis and fungal communities based on ITS analysis.

Site ID	OTU Number	Chao1	Shannon	Simpson	Richness	Raw
			Bacteria			
M30	1290 ± 27	1850.4 ± 123.1	8.58 ± 0.15	0.9916 ± 0.002	0.6971± 0.004	17,138
M24	1102 ± 42	1590.6 ± 111.5	7.82 ± 0.31	0.9809± 0.003	0.6928 ± 0.007	16,413
M27	1594 ± 21	2205.0 ± 126.1	8.97 ± 0.17	0.9935± 0.001	0.7229 ± 0.009	19,948
M19	1910 ± 24	2149.3 ± 118.4	9.32 ± 0.23	0.9941± 0.004	0.8887± 0.012	23,801
M3	1149 ± 15	1766.4 ± 117.3	8.56 ± 0.30	0.9932± 0.003	0.6504 ± 0.011	12,281
M8	1483 ± 23	2313.3 ± 124.4	9.2 ± 0.27	0.9959± 0.001	0.6411± 0.022	12,929
M9	1498 ± 19	2120.3 ± 119.8	8.86 ± 0.16	0.9947± 0.004	0.7065 ± 0.016	17,177
M39	1207 ± 11	1604.9 ± 111.2	8.83 ± 0.17	0.9935± 0.003	0,7521 ± 0.011	13,580
M29	1287 ± 16	1775.2 ± 121.1	8.90 ± 0.22	0.9952± 0.004	0.7250 ± 0.014	16,899
M31	1936 ± 22	2789.9 ± 125.4	9.93 ± 0.24	0.9976± 0.002	0.6939 ± 0.023	13,521
M4	2030 ± 26	3055.0 ± 128.6	9.96 ± 0.19	0.9978± 0.004	0.6645 ± 0.019	15,428
			Fungi			
M30	407 ± 3	444.0 ± 47.4	6.17 ± 0.11	0.9694 ± 0.004	0.9167 ± 0.006	19,346
M24	435 ± 5	476.6 ± 39.5	3.98 ± 0.23	0.7591 ± 0.002	0.9128 ± 0.008	20,937
M27	116 ± 2	139.0 ± 29.3	4.21 ± 0.24	0.8632 ± 0.001	0.8348 ± 0.005	60,281
M19	410 ± 5	422.2 ± 22.1	6.14 ± 0.33	0.9635 ± 0.003	0.9711 ± 0.007	23,848
M3	413 ± 2	438.4 ± 14.2	5.15 ± 0.18	0.9341± 0.002	0.9420 ± 0.005	13,908
M8	670 ± 6	705.6 ± 31.1	6.72 ± 0.17	0.9724 ± 0.004	0.9495 ± 0.008	16,541
M9	594 ± 6	651.0 ± 23.2	5.63 ± 0.19	0.9121 ± 0.001	0.9124 ± 0.007	23,872
M39	403 ± 7	414.1 ± 22.2	5.77 ± 0.27	0.9122 ± 0.003	0.9731 ± 0.007	14,049
M29	378 ± 2	417.1 ± 13.1	6.12 ± 0.31	0.9647 ± 0.003	0.9062 ± 0.008	10,035
M31	639 ± 9	705.1 ± 29.2	7.35 ± 0.25	0.9861 ± 0.003	0.9063 ± 0.005	16,505
M4	511 ± 4	575.1 ± 18.4	6.16 ± 0.23	0.9552 ± 0.005	0.8886 ± 0.004	29,912

4. Discussion

Arctic terrestrial ecosystems are the largest depositories of organic carbon, and the loss of their stability is becoming increasingly real in the light of ongoing climate change. Even a slight warming can lead to the involvement of a significant part of the organic matter buried in the Arctic in the carbon cycle, which will make these ecosystems the largest source of greenhouse gas methane (CH_4). At the same time, the activity and structure of microbial communities of these soils can be used to indicate changes under the influence of global warming and anthropogenic impact, as well as the effectiveness of the restoration of disturbed peat bogs in the permafrost zone. Taking into account the insufficient knowledge of the microbial communities of the cryosphere, the newly obtained data will significantly supplement the knowledge on the diversity of microorganisms and will allow us to assess their role in this ecosystem while taking into account the metabolic potential.

The soil cover and its functional characteristics of the permafrost-affected ecosystems of the Arctic and highlands has been investigated in only a few pedological and geophysical studies, which have mainly been devoted to undisturbed landscapes of palsa and polygon peatlands sites [36–38]. From the standpoint of methods of microbiological studies in this region, they have been limited to the application of conventional microbiological methods [39,40], and several works were performed based on modern molecular approaches [41]. In this study, we used a high-throughput next-generation sequencing approach, which

allowed us to significantly expand the understanding of microbial diversity. Generally, Arctic bacterial communities seem to be dominated by taxa differently than in other biomes, which likely reflects the impact of polar environmental conditions on microbial communities [42]. Tundra soils are generally characterized as poorly enriched by organic matter, saturated, and poorly aerated. These conditions result in relatively low levels of microbial diversity [43].

The integrative analysis of data on characteristics of the primary site is reflected in the matrix (Table 6).

Table 6. Matrix of habitats describing disturbance types along the initial landscape types. Abbreviations: green cells indicate less disturbed objects, yellow—more disturbed. The intensity of the color reflects the degree of severity of the property along the gradient.

Initial Microlandscape Type	Disturbances				Reference
	Areal—Self-Regeneration	Areal—Restoration Activities	Linear	Scattered	
Shallow peat tundra on slopes	M3	No sample	M31	M29	M24
Ombrotrophic shallow peatlands of flat landscapes	M19	No sample	No sample	M39	M30
Rich and transition fens in the lowlands and riverine habitats	M8	M9	M4	No sample	M27

The initial landscapes were rated along the resilience gradient from lower to higher resilience. Less resilient are slopes. The organic layer is, as a rule, thin and, due to the sandy bedrock, could be easily destroyed. The ombrotrophic mire micro-landscapes were resilient at the medium level. In contrast, the most resilient were peatlands at the lower positions, depressions, riverine habitats, etc. that were represented by poor and transition fens.

The revealed differences in the diversity of the bacterial communities prompted us to study mor deeply the differences in the taxonomic composition and relative abundance of bacterial taxa. Beta diversity was analyzed to elucidate major drivers of microbial community composition using the weighted UniFrac distance metric, which uses phylogenetic information to compare environmental samples. Non-metric multidimensional scaling (NMDS) indicated habitat type as the vector responsible for the greatest variability (Figure 5). Our results showed that the microbial communities of the studied sites demonstrated pronounced clustering for similar habitats types and were clearly separated from others.

The analysis of the relative representation of bacteria showed that a sharp decrease in the content of acidobacteria of the genus *Granulicella* and *Candidatus Solibacter* could indicate anthropogenic disturbance (Figure 6).

Data on the composition of methanotrophs and closely related trophic methylotrophic microorganisms can also be an important indicator of disturbance. Methanotrophic Proteobacteria were not found in the studied samples of undisturbed objects. Methylacidiphilaceae are probably responsible for methane oxidation in these soils (Figure 7). Their high proportion was also registered on the disturbed slopes, where permafrost thaw is very intensive [24].

At the same time, methanotrophic Proteobacteria *Methylobacterium-Methylorubrum* and facultatively methylotrophic psychrotolerant bacterium *Methylorosula* were detected only in the anthropogenically disturbed objects, and the proportion of methanotrophic verrucomicrobia was significantly lower. The data obtained convincingly indicate changes in microbial communities of the methane cycle under anthropogenic influences, which allows us to consider them as indicators for environmental monitoring.

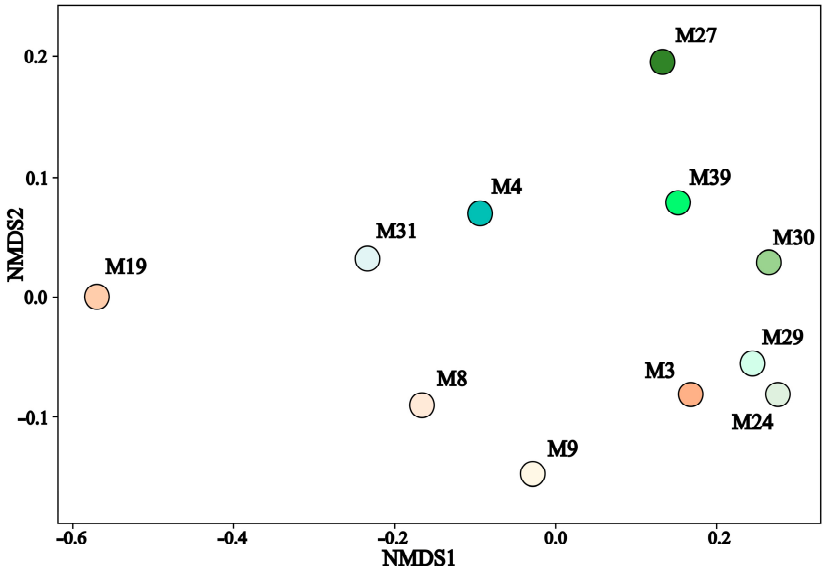

Figure 5. Non-metric multidimensional scaling (NMDS) plot based on the Bray–Curtis similarity coefficients of experimental soil samples. Points closer to one another in ordination space are more similar than those apart.

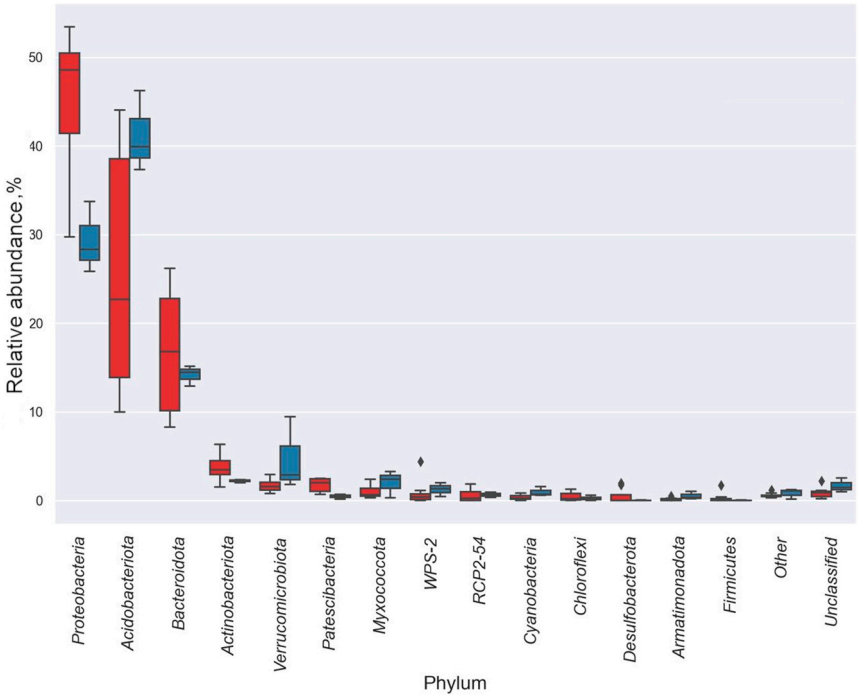

Figure 6. Comparison of the bacterial community structure at the phylum level across all studied sites. Abbreviations: blue boxplots—undisturbed sites, red boxplots—disturbed sites; outliers in a data set are indicated by the blue rhombus. See Table 6 for details.

Numerous natural and anthropogenic factors influence soil microbial communities, and these factors must be considered when interpreting microbiome parameters. In this work, we discuss only some of these factors. It should be noted, however, that, in the current study, climatic parameters (such as temperature and precipitation), which can significantly affect the soil microbial community, probably did not impact microbial diversity. This is because all study sites were chosen in the same bioclimatic region and were, therefore, exposed to similar weather conditions.

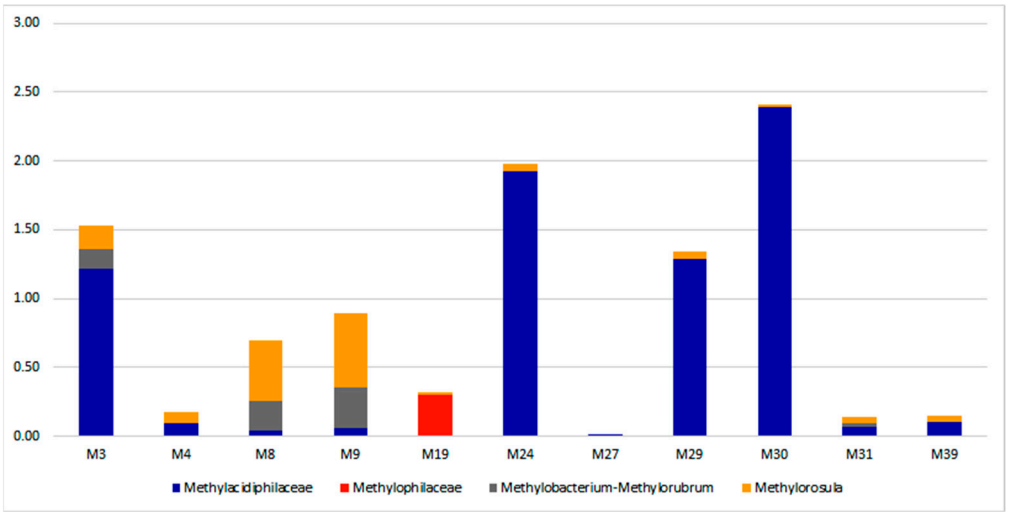

Figure 7. Distribution patterns of methylotrophs retrieved from the peat samples.

5. Conclusions

Intact and disturbed permafrost-affected Arctic shallow peat soils are diverse in terms of microbial life. Our research demonstrated that, despite very harsh Arctic environments, the diversity of its soil bacterial communities is as high as in other biomes. Anthropogenic disturbance leads to a significant decrease in the representation of Acidobacteria and Verrucomicrobia, while the proportion and diversity of Proteobacteria increase. Fungal communities in undisturbed sites may be characterized as monodominant, and anthropogenic impact leads to an increase in fungal diversity. The patterns of changes depend on the initial landscape and level of disturbance. The usual proteobacterial methanotrophs were not detected in undisturbed reference sites, while *Methylobacterium-Methylorubrum* associated with methylotrophic *Methylorosula* were found in the disturbed sites.

Author Contributions: Conceptualization, methodology, I.K., T.M. and A.S.; resources, T.M. and A.S.; formal analysis, I.K., D.G. and M.S.; writing—original draft preparation, I.K. and T.M.; writing—review and editing, I.K., T.M. and A.S.; visualization, I.K. and T.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw data generated from 16S rRNA sequencing of microbial communities have been deposited in the NCBI Sequence Read Archive (SRA) under the accession numbers SRX18380871–SRX18380881 (Bioproject PRJNA904242).

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Conflicts of Interest: The authors declare no conflict of interest.

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