

Article

Soil Microbiome of Abandoned Plaggic Podzol of Different-Aged Fallow Lands and Native Podzol in South Taiga (Leningrad Region)

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Abstract: The soil microbiome is composed of various communities that play an important role in the existence of ecosystem services and the sustainable functioning of ecosystems under high anthropogenic loads. The transition of soils to a fallow state and their subsequent transformation lead to a notable alteration in the taxonomic composition of the soil microbiome, impacting the biochemical processes within the soil and its fertility levels. The object of this study comprised different-aged fallow soils of the southern taiga in the vicinity of Ban'kovo village, Leningrad region. The method comprising the high-throughput sequencing of 16S rRNA gene fragments using an Illumina MiSeq sequencer was used to analyze the microbial community. The general processing of sequences was carried out with the dada2 (v1.14.1) package. It was found that the morphological organization of fallow soils has significant differences from the native podzol. In fallow soils, there are signs of leaching expressed in the accumulation of leached mineral particles, which indicates the degradation of the fallow–arable horizon. At the same time, there is a decrease in the content of P₂O₅ and K₂O and an increase in the content of N-NH₄ and N-NO₃ in fallow soil. The analysis of alpha diversity index values showed that the highest level of alpha diversity in the microbial community is characteristic of 40-year-old soil, the alpha diversity index decreased with the increasing time of the fallow state, and the lowest alpha diversity index was observed in the native podzol. According to the values of the beta diversity index, a high correlation between the soil microbiome and the physicochemical characteristics of the soil was revealed, which indicates the formation of functional specialization in the studied microbial communities. As a result of the study of the taxonomic composition of microbial communities in fallow soils, it was found that the most represented microbial communities in fallow soils belong to Nitrosomonadaceae (Pseudomonadota), Mycobacterium (Actinobacteria), Nitrospira (Nitrospirota), and Luteolibacter (Verrucomicrobiota). The duration of post-agrogenic transformation is the leading factor influencing the changes in microbial communities; so, with an increase in the time that soils were in a fallow state, an increase in the oligotrophic microbial community was observed.

Keywords: 16S amplicons; high-throughput sequencing; ecogenesis; podzols



Citation: Lavrishchev, A.; Litvinovich, A.; Abakumov, E.; Kimeklis, A.; Gladkov, G.; Andronov, E.; Polyakov, V. Soil Microbiome of Abandoned Plaggic Podzol of Different-Aged Fallow Lands and Native Podzol in South Taiga (Leningrad Region). *Agronomy* **2024**, *14*, 429. <https://doi.org/10.3390/agronomy14030429>

Academic Editor: Chengsheng Zhang

Received: 24 January 2024

Revised: 18 February 2024

Accepted: 18 February 2024

Published: 22 February 2024



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1. Introduction

According to UN calculations, the world population by 2050 will be about 10 billion people [1]. This will lead to a significant shortage of food and water; to provide for such a population, an increase in food production of 70% will be necessary. The increased production of agricultural products will have a negative impact in the form of land degradation,

the depletion of natural resources, and environmental pollution through the increased application of mineral and organic fertilizers to the soil [2]. Therefore, one of the effective solutions to reduce environmental impacts is the use of the soil microbiome, which is involved in the regulation of biochemical processes and fertility levels [3]. Thus, the use of the soil microbiome can be considered the most beneficial and long-term solution to the problems of soil degradation, fertilizer pollution, and food security [4]. Agricultural land development fundamentally alters the soil microbiome and carries long-term negative consequences for ecosystem productivity [5]. Long-term monoculture cultivation can lead to the accumulation of soil pathogens, reducing plant resistance against insect pests [6]. The composition of the soil microbiome is important for soil and plant health, food safety, and crop productivity [7]. Hannula et al. [8] suggested that soil transfer to a fallow state increases the resistance of grassland plant communities to insect herbivores. With climate change and rising global temperatures, the soil microbiome may undergo major structural and functional transformations [9]; changes in the soil microbiome composition may affect biogeochemical cycling and carbon sequestration from the atmosphere, therefore transforming soils from a sink to a source of carbon dioxide [9]. Boreal forest soils store a large amount of carbon and play a crucial role in the planet's carbon balance.

To date, the area of agricultural land has decreased by 2.2 million km² during the 20th century, which was converted to a fallow state and transformed by natural and anthropogenic processes of soil formation, forestation, waterlogging, etc. [10,11]. Russia tops the list of countries in terms of the share of abandoned agricultural land [10]. Various socio-cultural changes in the country at the end of the 20th century led to an uncontrolled transfer of agricultural soils to a fallow state [12]. The Northwestern region of Russia is characterized by a long history of development, starting from the time of the ancient Slavs [13], which allows us to consider different stages of soil transformation and the soil microbiome depending on their transfer to fallow land. Due to the huge area of fallow lands, it is necessary to understand the direction of transformation of these lands and the ecosystems formed on them, as these areas are richer in microbial communities, contents of biogenic elements, and plant biodiversity. Studies by Luri et al. [14] show that post-agrogenic soils in the taiga zone develop in the direction of native podzols, but, depending on local lithological features, Rendzinas and Rendzic Leptosols are formed on carbonate rocks [15], and Retisols and Gleysols are formed on clayey rocks, so the process of transformation into natural soils may take different amounts of time. Thus, in podzols formed on oligomictic sands, after 20 years, signs of leaching can already be determined [12], while, in polymictic sands, the process of podzolization will develop very slowly. In addition to morphological changes in soil as a result of the transition to fallow land, significant carbon losses from 64 to 870 Tg are observed as a result of soil degradation [10]. The greatest humus losses were recorded in semi-humid and arid areas, which initially had high fertility [16]. On the other hand, the cultivation of soils in the taiga zone resulted in the formation of thick arable soils with a pronounced humus-accumulative horizon [17]. At the initial stages of soil transition to a fallow state in the boreal zone, there is an increase in the process of carbon sequestration; this is associated with the formation of woody vegetation and shrubs, but carbon sequestration differs depending on the changes in environmental parameters, namely, the quality of litter, soil temperature, moisture, and microbial composition [18]. The aim of this work is to study the evolutionary dynamics of the microbiome of different-aged fallow soils in the Leningrad region. In this regard, the following tasks were set:

- To study the morphometric features of soil transformation under the conditions of the post-agrogenic development of ecosystems;
- To determine the physico-chemical parameters of different-aged fallow soils;
- To analyze the taxonomic and functional diversity of the microbiome of different-aged (40-, 80-, and 120-year-old) fallow and native soils using modern methods including high-throughput sequencing and bioinformatic analysis.

2. Materials and Methods

2.1. The Study Area

The study area is located at the end of the moraine zone of the Valdai glaciation. The parent materials are red–brown blocky loams of moraine plains, where the moraine is overlapped by fluviglacial sandy loam [19]. The area is characterized by favorable agroclimatic conditions, rather mild and short winters, a long, warm vegetation season, and moderate precipitation. This research was conducted in the vicinity of Ban'kovo village, Leningrad region, Russia (Figure 1). The site belongs to the southern taiga bioclimatic zone, and umbric podzol residual-calcareous predominate in the area [20]. The vegetation community is represented by coniferous forest with an admixture of small-leaved species (birch, aspen).

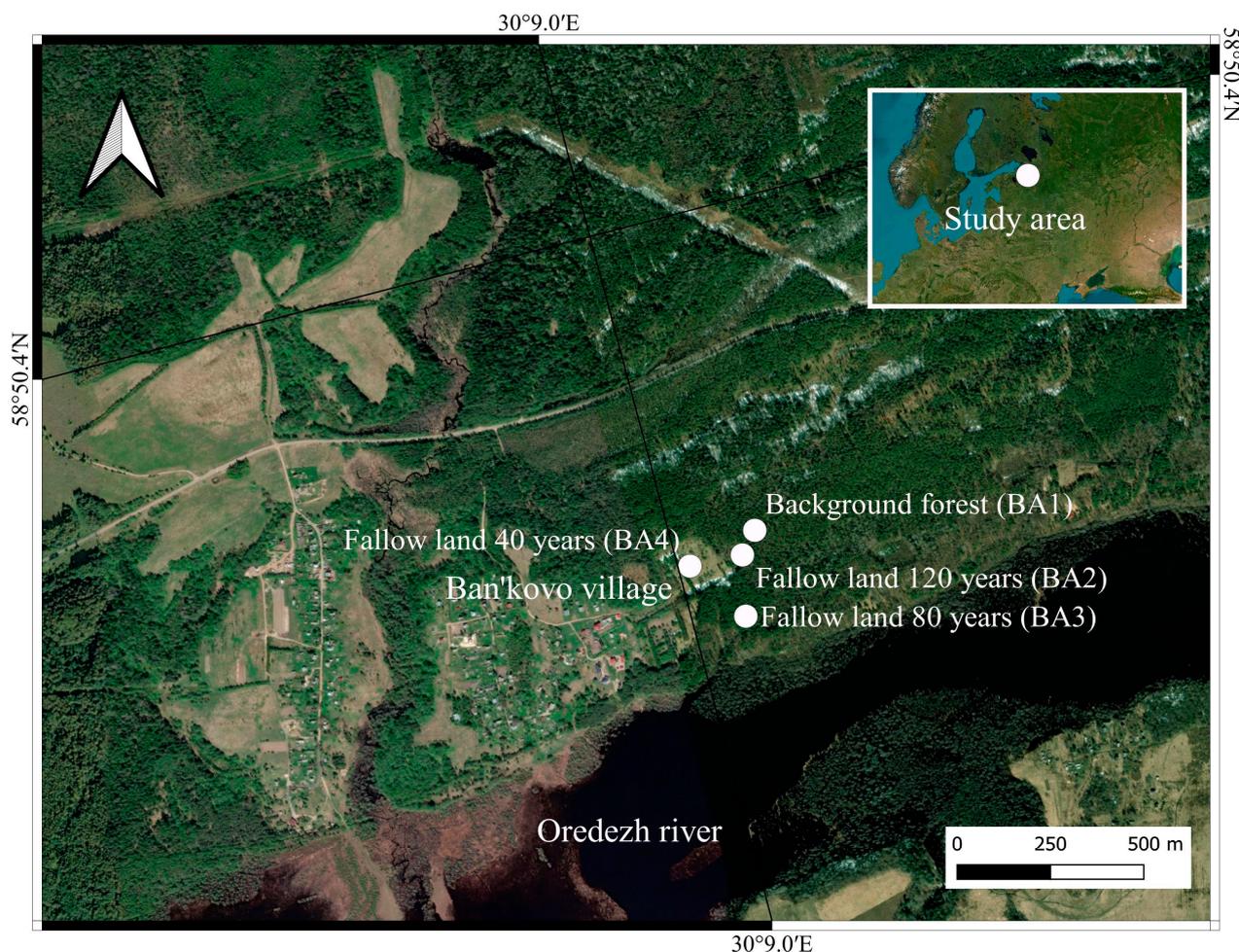


Figure 1. The study area. Ban'kovo village, Leningrad region, Russia.

Fallow soils aged 40, 80, and 120 years old, as well as natural podzol, were investigated. All the studied soils were formed on the same type of soil-forming rocks, on blocky sandy loams of water-glacial origin, underlain at a depth of 70–80 cm by red–brown moraine loams. Soil profiles are shown in Figure 2.

The upper humus-accumulative horizons of soils were used to analyze the soil microbiome. A description of the studied horizons is presented in Table 1.

The soil microbiological samples were frozen at the time of collection and delivered to the Applied Ecology Laboratory of Saint-Petersburg State University. The samples for chemical analyses were stored at +4 °C to analyze the main agrochemical parameters and pH. The soil was grounded and passed through a 2 mm sieve to obtain a fine earth fraction. For all the samples, the main nutrition parameters were determined—pH, available phosphorus and potassium, ammonium, and nitrate nitrogen.

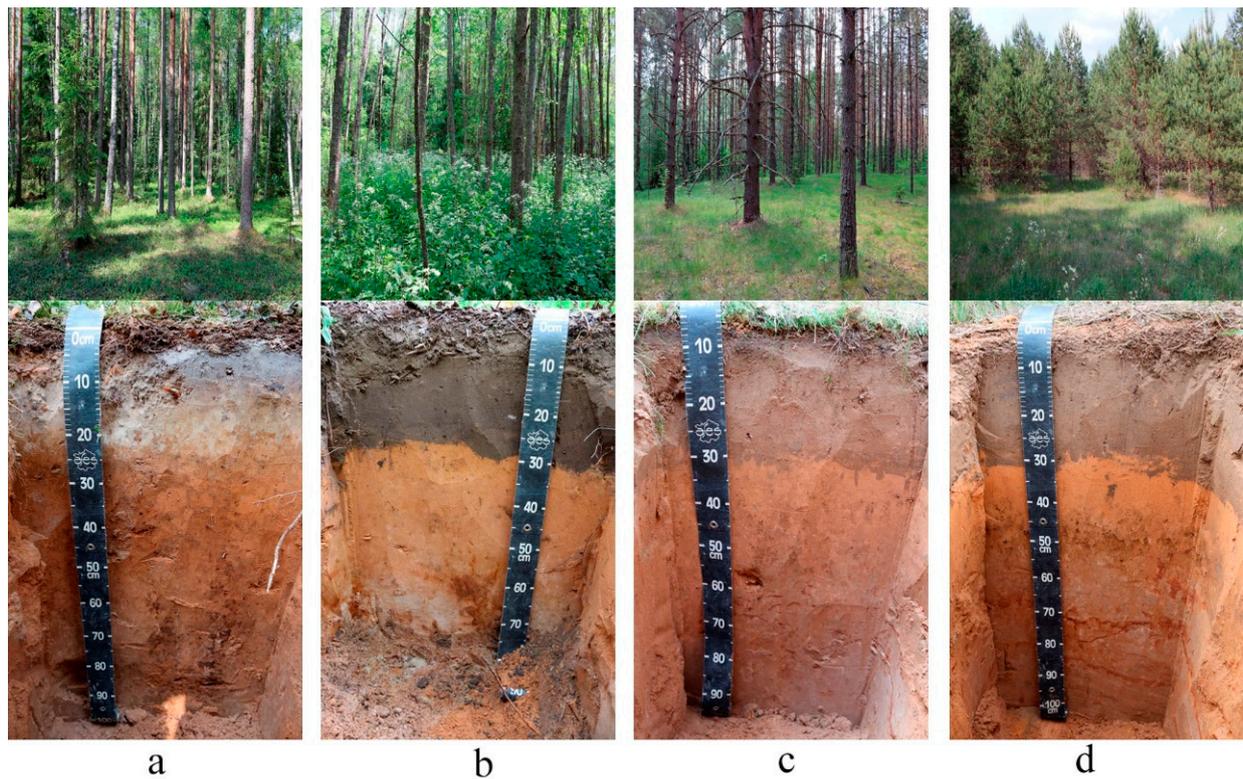


Figure 2. Studied soils in the vicinity of Ban'kovo village. (a)—native stagnic podzol (arenic) on loam binary deposits (BA1), (b)—120-year-old plaggic podzol (arenic) on loam binary deposits (BA2), (c)—80-year-old plaggic podzol (arenic) on loam binary deposits (BA3), and (d)—40-year-old plaggic podzol (arenic) on loam binary deposits (BA4).

Table 1. A description of studied soils.

Soil ID	Horizon *	Depth, cm	Description	Color	Location	Coordinates	Soil Name **
BA1	Oe	0–4	Moss cover, poorly decomposed Mineral horizon	-	Benchmark forest	N 58.832090 E 30.153881	Stagnic podzol (arenic)
	E	4–10	characterized by the loss of silicate clay, iron, and aluminum	2.5YR 7/1			
BA2	O	0–3	Moss cover, slightly decomposed Postagrogenic horizon, sandy loam, accumulation of leached mineral grains,	-	Fallow land, 120 years old	N 58.831588 E 30.153031	Plaggic podzol (arenic)
	Ahp	3–28	abundance of roots, border uneven in color Postagrogenic horizon, sandy loam, the accumulation of leached mineral grains, abundance of roots, border uneven in color	5YR 5/4			
BA3	Ahp	0–30	Postagrogenic horizon, sandy loam, abundance of roots, the accumulation of leached mineral grains, border uneven in color	5YR 6/4	Fallow land, 80 years old	N 58.830129 E 30.152454	Plaggic podzol (arenic)
BA4	Ahp	0–30	Postagrogenic horizon, sandy loam, abundance of roots, the accumulation of leached mineral grains, border uneven in color	5YR 6/4	Fallow land, 40 years old	N 58.831647 E 30.150548	Plaggic podzol (arenic)
	Bs	30–70	Horizon with illuvial accumulation of sesquioxides	5YR 6/6			

* Guidelines for soil description [21]; ** World reference base for soil [22].

2.2. Laboratory Analyses

The pH values were determined using a pH analyzer by the potentiometric method. The carbon content was determined on a CHN analyzer (EA3028-HT EuroVector, Pravia, PV, Italy). The particle size distribution of the samples was determined by the sedimentation method [23]. The agrochemical characteristics, mobile phosphorus, and potassium were determined using standard procedures according to the national Russian standard GOST R 54650-2011 [24], which is based on extracting mobile phosphorus (P_2O_5) and potassium (K_2O) compounds from the soil with a hydrochloric acid solution. Available forms of nitrogen were determined according to ISO 14256-1 [25], which first extracts exchangeable ammonium (NH_4^+) from the soil with a potassium chloride solution and then photometrically measures the colored solution.

For the microbiome analysis, the upper horizon of each soil was chosen. DNA was isolated from six soil samples in four replicates using the MN FastDNA Spin Kit (MP Biomedicals, Eschwege, Germany) using a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). The quality control of the isolation was carried out by PCR (polymerase chain reaction) and agarose gel electrophoresis.

To evaluate the microbial content in the soil the real-time PCR of the 16S rRNA fragment for bacteria was performed on the CFX96 Real-Time PCR Detection System (Bio-Rad, Feldkirchen, Germany) using primers EUB338 5'-ACTCCTACGGGAGGCAGCAG-3' [26] and EUB518 5'-ATTACCGCGGCTGCTGG-3' [27], as described previously [28]. The threshold cycle (CT) data were converted to the number of ribosomal operons per 1 g of soil. The significance of the mean differences between soil horizons of measurement was calculated using an ANOVA with the Tukey HSD test [29].

Due to the well-developed methods of taxonomical annotation and relatively representative sequencing, the v4 variable region (f515/r806) of the 16S rDNA gene was selected for future analysis. The sequencing of the variable region was performed on the Illumina MiSEQ sequencer using primers f515 (GTGCCAGCMGCCGCGGTAA) and r806 (GGA CTACVSGGGTATCTAAT) [30]. The general processing of sequences was carried out using the dada2 (v1.14.1) package [31]. Reads were filtered by length (240 bp for forwards and 180 for reverse), and for the expected error rate ($\text{maxEE} = 2$), no N was allowed. Reads were paired by the "consensus" method and annotated using the Bayesian Naive classifier using the SILVA 138 database as the training set [32]. The main diversity analysis of the results was carried out using the phyloseq (v1.30.0) package in R (v3.6.3) [33]. The microbiome composition of the soil was connected with the variability in its chemical characteristics using Canonical Correspondence Analysis (CCA) in vegan [34].

3. Results and Discussion

3.1. Features of the Transformation of the Soils as a Result of Their Transfer to the Fallow State

The morphological organization of the soils of different ages (40, 80, 120 years) of fallow lands has weak differences and is expressed in the change in soil color and occurrence of illuvial accumulation of sesquioxide processes. The soils of the 120-year-old fallow lands are characterized by a dark-colored organo-accumulative fallow-arable horizon, with signs of leaching shown in the accumulation of leached mineral grains. In the lower part of the profile, we can note the signs of accumulation of sesquioxides, which indicates a decrease in the mobility of organo-mineral compounds as a result of changes in soil acidity. The absence of a podzol horizon may be related to the material and mineralogical composition of fluvioglacial deposits. The 80-year-old soil also had signs of the accumulation of leached mineral particles in the arable horizon. The lower part of the profile is characterized by less pronounced processes of accumulation of sesquioxides in the soil, which may be related to the absence of water stagnation. As well as in the soils of the 80- and 120-year-old fallow lands, the accumulation of leached mineral grains was observed in the 40-year-old fallow soils, and the underlying illuvial horizon was more pronounced. Several boundaries of topsoil are clearly observed, which is due to the fact that the soil is located on a relatively level area in relation to the soils of the 80- and 120-year-old fallow soils. The natural podzol

is characterized by the development of a thick podzol horizon, which is 20 cm. Thus, the analysis of the morphological organization of the soils revealed the following features: old arable horizons were characterized by the accumulation of leached mineral grains, the formation of a natural podzol horizon was not noted, and the formation of podzols can take up to 300 years [35]. At the same time, in the lower part, there are still signs of the accumulation of sesquioxides, and the intensity of this process is influenced by the soil location in the relief and the proximity of the groundwater table.

Data on the chemical composition of the soils are summarized in Table 2.

Table 2. Physico-chemical parameters of the studied soils. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits.

Soil ID	Horizon	pH _{H₂O}	pH _{KCl}	OC %	N-NH ₄ ⁺ Available Form, mg·kg ⁻¹	N-NO ₃ ⁻	P	K	Particle Size Distribution		
									Clay, %	Silt, %	Sand, %
BA1	Oe	4.22	2.24	43.7	90.07	11.18	250	158	-	-	-
	E	4.98	2.43	1.04	3.88	0.36	29	25	4	4	92
BA2	Oe	5.85	4.76	4.70	9.43	2.12	205	28	-	-	-
	Ahp	5.64	5.17	1.39	2.46	0.45	140	23	5	7	88
BA3	Ahp	5.68	4.17	0.62	1.36	0.36	222	505	3	4	93
	Ahp	5.89	4.33	1.44	1.1	0.36	276	397	4	7	89
BA4	Ahp	5.89	4.33	1.44	1.1	0.36	276	397	4	7	89
	Bs	5.85	4.55	0.38	0.68	0.18	106	522	3	7	90

The soils are characterized by a relatively high level of acidity. Soil cultivation has led to a significant increase in the carbon content of organic compounds in the arable horizons, where it remains relatively high even in the old arable horizons. The carbon content in all the studied soils decreases with depth, which is generally characteristic of the soils of the bioclimatic zone. At the same time, we can note that the carbon content in the 80-year-old fallow soils is the lowest among the studied fallow soils, as well as the occurrence of the illuvial accumulation of the sesquioxide process. In the 120-year-old soil, as a result of forest ecosystem development, there is an active accumulation of organic carbon as forest litter. In the 120-year-old fallow soil, the highest level of ammonium and nitrate nitrogen content was observed, indicating an active process of atmospheric nitrogen fixation by soil microorganisms from the atmosphere. At the same time, the 120-year-old fallow soil lost more than half of the content of exchangeable nutrition elements (P₂O₅, K₂O), while in the 40- and 80-year-old soils, it remains quite high. This indicates that although there are no significant morphological attributive changes in the 120-year-old soil, chemical changes related to nutrient element removal are already taking place. The tendency for nutrient element content in soils to decrease with the age of the fallow state was also noted by Litvinovich et al. [36]. All the investigated soils belong to sandy loam with a predominance of medium-sized sand fraction.

3.2. The Soil Microbiome of Different-Aged Fallow Lands in the Vicinity of Ban'kovo Village, Leningrad Region

The 16S rRNA gene libraries were obtained for fallow and native soil. The bacterial operons were quantified using quantitative PCR in different horizons of different chronosequence stages. Although the number of bacterial operons decreased between horizons of the same point with increasing depth, it was not significantly different between arable horizons and its counterparts between chronoserries (Figure 3).

The greatest number of bacterial operons was observed in forest litter microbiomes, which is consistent with the results of the agrochemical analysis—in the upper horizons, the concentration of the available forms of nitrogen and carbon is maximum, which leads to an increase in the number of microbiota.

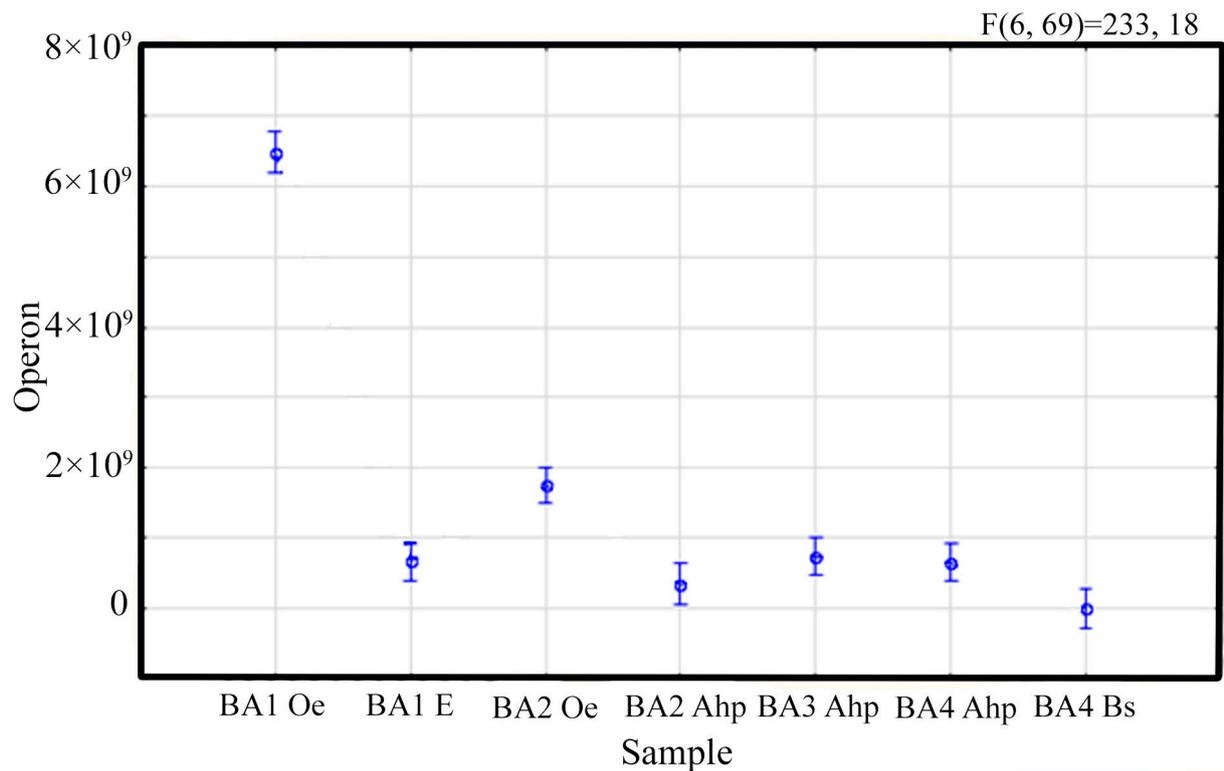


Figure 3. Number of bacterial operons per 1 g of soil at different points in the plaggic podzol chronoserries. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits. Significant differences between localities $p < 0.001$.

The results of the alpha diversity indices of the soil microbiome show a taxonomic composition of the microbiome (Figure 4). If we compare them between the fallow–arable horizons of the soils and the podzol horizon of the native soil, it appears that the younger soils have a greater richness of communities, which decreases with time after the transition to the fallows state. At the same time, the forest litter of the native soil, in which, according to the quantitative analysis, the maximum number of bacterial operons was observed, had minimal richness. The alpha diversity index of the soil microbiome in the 40-year-old fallow soil is several times higher than in the background soil. This indicates a high level of stability in fallow communities relative to the native soils. Similar results were obtained for the soils of agricultural fields under the fallow state in 1–5 years; here, an increase in organic matter as well as nutrients was observed [37,38]. The high alpha-diversity index of the soil microbiome of fallow land may indicate the process of soil transformation. Thus, the transformation of soils of fallow land results in a gradual shift in soil microbiome composition toward natural ecosystems.

The analysis of beta diversity parameters of the chronoserries of the fallow soils did not reveal a clear pattern in the differences associated with different stages of the fallow state (Figure 5). The most pronounced differences are shown on the NMDS1 axis, which illustrates the difference between zonal podzol and fallow soils. The NMDS2 axis reveals a trend in the difference in the soil microbiomes between different soil horizons. At the same time, different horizons from the same soil profile have a tendency to cluster with each other. Based on the analysis, we can conclude that agriculture has a strong influence on the composition of the soil microbiome: even after 120 years, the soil microbiome was not identical to the natural one.

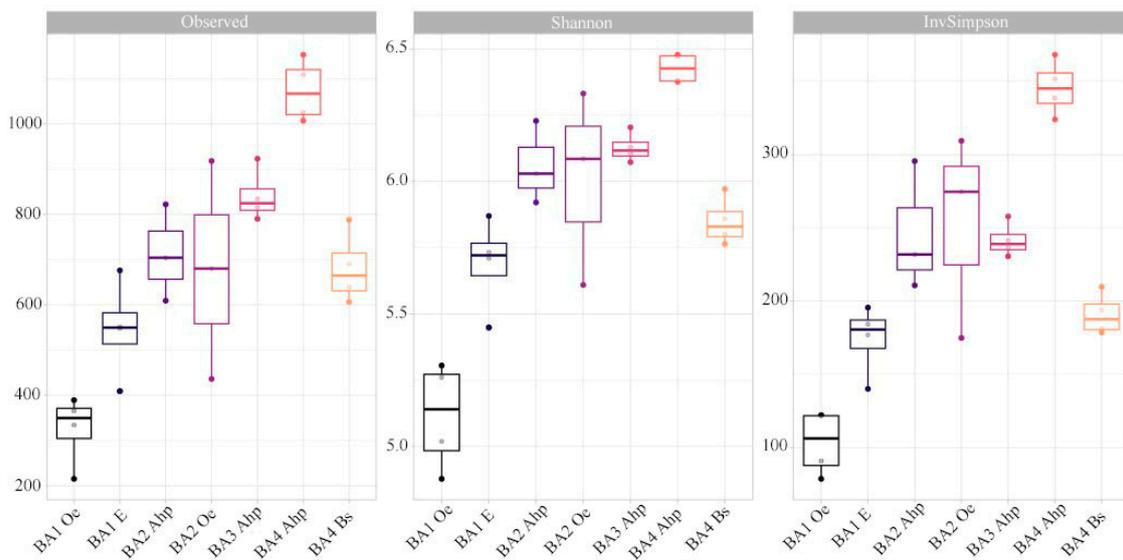


Figure 4. Alpha diversity of microbiomes in fallow and native soils in the vicinity of Ban'kovo village, Leningrad region. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits.

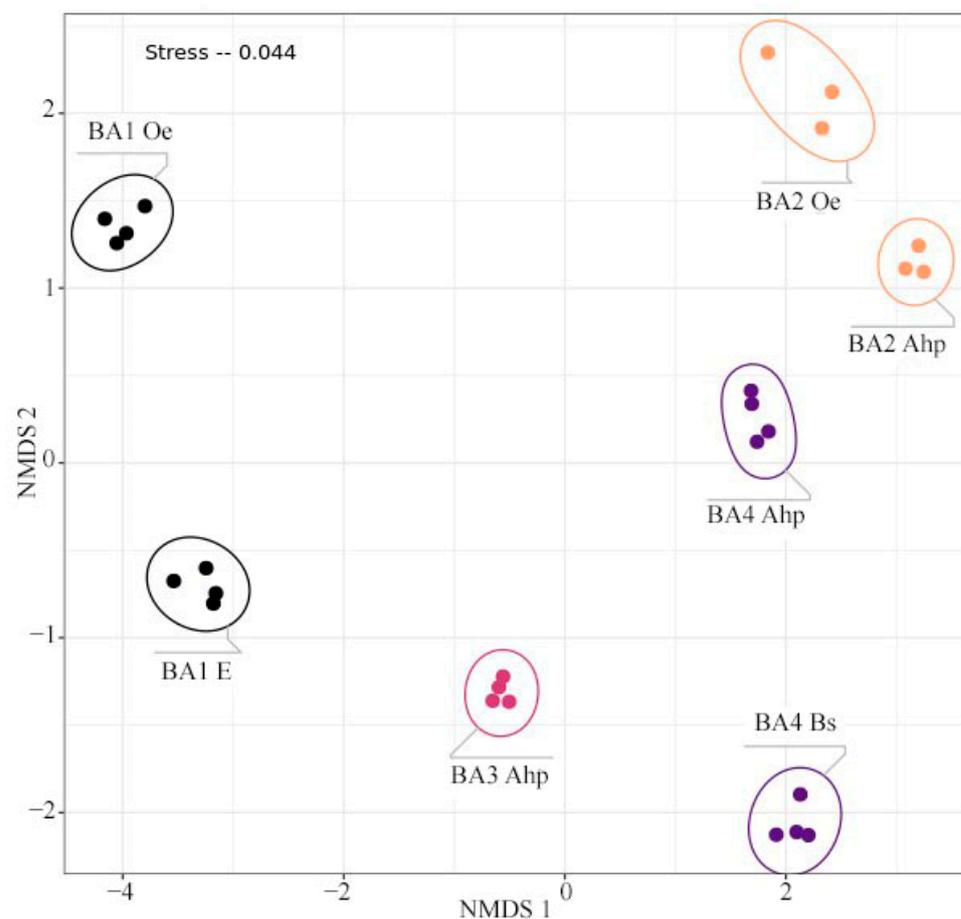


Figure 5. Beta diversity of microbiomes in fallow soils in the vicinity of Ban'kovo village, Leningrad region. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits.

According to the analysis of the beta diversity index, a high correlation between the soil microbiome of the studied soils and the physicochemical characteristics of the soils was revealed. It seems that there was a deep functional specialization of the studied communities despite the close location of sampling sites. This is shown by the fact that the common prokaryotic core is practically absent for the upper soil horizons (Figure 6), which is not typical for similar data (close sampling points, common ecotopes). As expected for the forest litter horizon, in which the highest number of operons was observed, the amount of available nitrogen and organic matter in the soil plays a key role in the formation of the microbiome. During the transformation of soils to the fallow state, we can note the contribution of different nutrients explaining the diversification of the microbiome. The microbiome of mature soil was characterized by a negative correlation between potassium content and soil acidity, which is due to the biochemical stability of mature soil in relation to fallow soils.

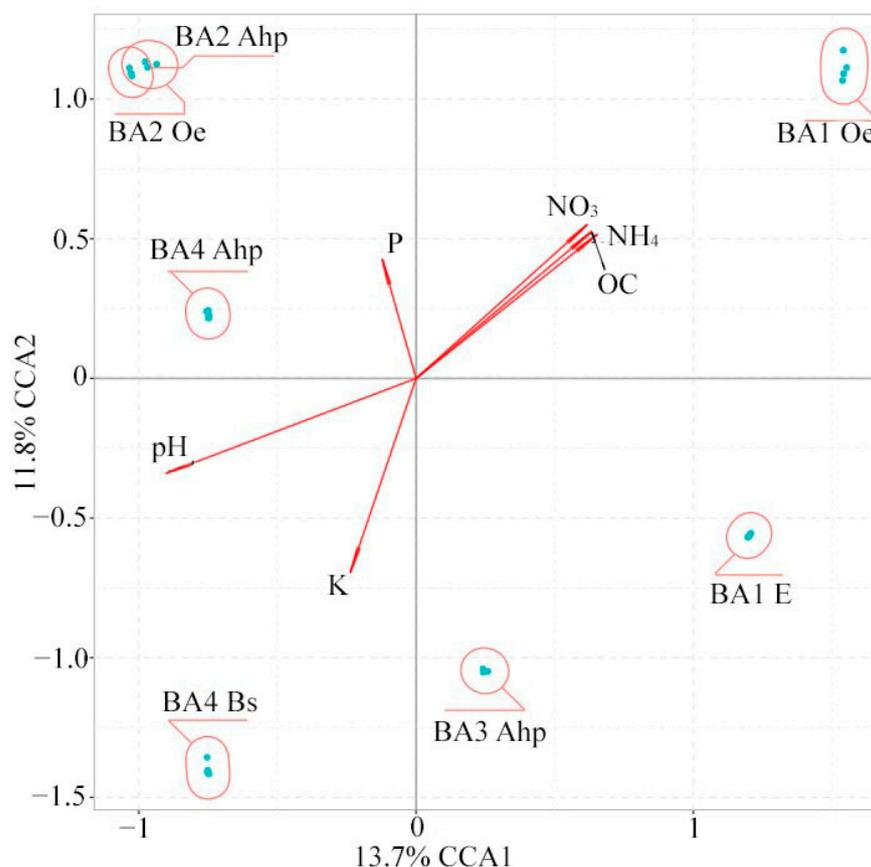


Figure 6. Canonical correlation analysis (CCA) plot showing the relationship between soil microbiome diversity and soil physicochemical parameters. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits.

The distribution diagram of core phylotypes in the studied soils is presented in Figure 7. In Figure 7, we see that the microbiome of the soils has few similarities among the studied samples, even considering that the sampling points are relatively close to each other. The obtained data show the general heterogeneity in the fallow soils and their high biological potential.

The studied soils near the village of Ban'kovo are represented by a high taxonomic level of phylotypes of Actinobacteria and Gammaproteobacteria and representatives of Bacteroidota, Verrucomicrobiota, and Planctomycetota. According to the ANCOM-BC analysis, only 21 phylotypes jointly represented at multiple locations showed significant changes in the chronosequence trend (Figure 8).

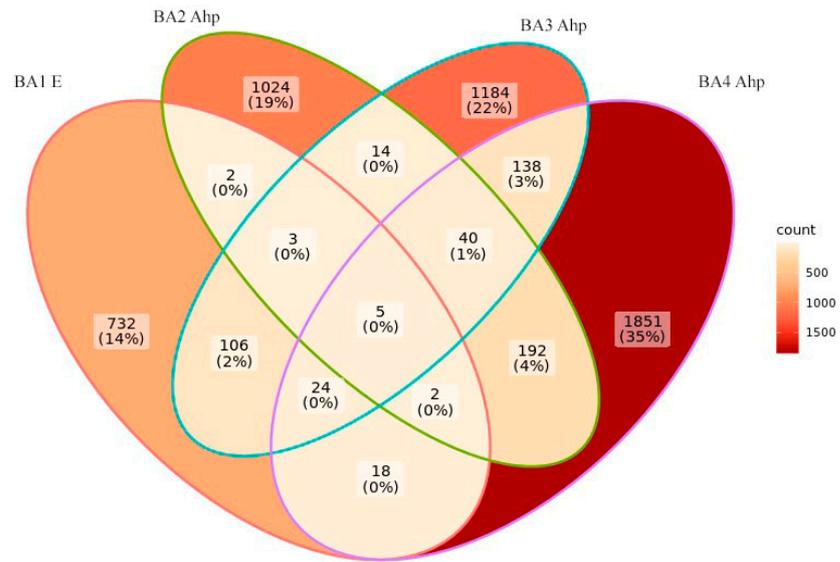


Figure 7. Core phlotypes between fallow soils. The analysis was prepared for the upper organomineral horizons (BA1 E, BA2 Ahp, BA3 Ahp, and BA4 Ahp). BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-year-old plaggic podzol (arenic) on loam binary deposits.

	benchmark		120		80	40	
Acidobacteriota-	25.2	33.3	15	15.1	31.5	21.1	17.8
Gammaproteobacteria-	12	6.8	13.6	18.8	11.1	10	10.3
Alphaproteobacteria-	13.1	12	12.2	13	12.1	10.2	6.7
Verrucomicrobiota-	6.7	12.9	4.6	9.6	13.3	14.7	13.5
Bacteroidota-	16.4	1.9	7.2	16	3.9	9.1	1.7
Actinobacteriota-	11.7	5	3.9	6.5	6.3	6.2	7.7
Planctomycetota-	8.2	10.6	4.8	6	6.5	7.2	3.5
Chloroflexota-	0	1.6	4.6	2.2	5.9	3.5	11.4
Patescibacteria-	0.4	0.4	5.8	1.8	1.2	3.5	4.7
Gemmatimonadota-	0	0.4	4.1	1.6	1.3	3.5	4.8
Myxococcota-	0.6	0.7	2.1	2.4	1.2	1.8	1.7
RCP2-54-	0.7	5	0.7	0.5	0.7	0.5	1.5
Thermoproteota-	0.1	3.3	0.8	0.2	0.9	0.6	3.2
Remaining taxa (99)-	4.8	6.2	20.7	6.3	4.1	8	11.5
	BA1 Oe	BA1 E	BA2 Ahp	BA2 Oe	BA3 Ahp	BA4 Ahp	BA4 Bs

Figure 8. The most represented phyla in the microbial community of the studied soils. BA1—native stagnic podzol (arenic) on loam binary deposits, BA2—120-year-old plaggic podzol (arenic) on loam binary deposits, BA3—80-year-old plaggic podzol (arenic) on loam binary deposits, BA4—40-years-old plaggic podzol (arenic) on loam binary deposits.

The most represented phylotypes belong to Nitrosomonadaceae (Pseudomonadota), Mycobacterium (Actinobacteria), Nitrospira (Nitrospirota), and Luteolibacter (Verrucomicrobiota) and unidentified representatives of the phyla Methylophilota and Actinobacteriota. Nitrosomonadaceae (Pseudomonadota) as well as Nitrospira (Nitrospirota) play an important role in the soil nitrogen cycle and are known as phylotypes that convert ammonia into nitrite through the process of nitrification, which is an essential step in the nitrogen cycle [39]. Luteolibacter (Verrucomicrobiota) is an essential phylotype for soil health and ecosystem service provision. These bacteria support soil productivity by encouraging nutrient cycling and the transformation of organic matter. Moreover, Luteolibacter (Verrucomicrobiota) has been discovered to generate secondary compounds with diverse biological functions such as antibiotics and promoters of plant growth. Their presence in the soil can improve plant development, increase yield, and enhance plant resistance to environmental stresses [40]. The taxonomic composition of the microbial community of the native soil showed differences between the forest litter consisting of undecomposed and partially decomposed plant remnants and the mineral podzol horizon. Thus, it was found that the forest litter horizon is characterized by an abundance of Bacteroidota and Actinobacteriota, and the proportion of phylotypes attributed to oligotrophs increases [41]. The same results were observed in the 120-year-old fallow soil, where oligotrophic organisms (k-strategists) predominate in horizons enriched by available organic matter and increased acidity. Acidobacteriota, Verrucomicrobiota, and Planctomycetota reacted to the decrease in available organic matter and nitrogen in the soil, and an increase in their abundance was observed; these representatives are characteristic of podzols with an acidic reaction [42]. As the content of available organic compounds decreases, the proportion of Acidobacteria in the community structure increases, which was also confirmed in the study by Górska et al. [43]. The composition of microbial communities of fallow soils was considerably different than that of the native soil, and differences were also observed within the fallow soils. In the soil of the 40-year-old fallow land, a predominance of Acidobacteriota, Verrucomicrobiota, and Chlorflexota was observed. The maximum abundance of Chlorflexota representatives was observed, which may be related to the relatively large amount of fresh organic matter and the fact that this group of microorganisms participates in its transformation [44]. The 80-year-old soil had the greatest similarity in the taxonomic composition of the microbial community with the native soil, possibly due to the relatively low content of available nitrogen and organic matter in the mineral soil horizons. However, the taxonomic composition of the 120-year-old soil differed from the native soil, with the domination of the same phyla of the microbial community (Acidobacteriota, Gammaproteobacteria, Alphaproteobacteria, Verrucomicrobiota), with a rather large proportion of Bacteroidota in the fallow–arable horizon, indicating a relatively high content of organic matter and nitrogen in the soil. These bacterial representatives are capable of decomposing complex organic matter [45]. However, in the young fallow soil, the predominance of Chlorflexota was observed, and its abundance decreased with increasing time in the fallow state. The results obtained are in agreement with the CCA results, and the association between these microorganisms and anaerobic communities is consistent with the change in aeration regime and soil microstructure in fallow soils of different ages. The presence of Patescibacteria, Gemmatimonadota, and Myxococcota phyla was observed in all the fallow soils; however, they were almost absent in the native soil, which indicates that fallow soils have more ecological functions compared with undisturbed soil. The increased abundance of the Myxococcota phylum may indicate the intensification of the turnover of carbon-containing matter because the representatives of this phylum are micro-predators that participate in the destruction of bacteria, eukaryotic organisms and are able to participate in the transformation of complex organic macromolecules [46]. Representatives of the phylum Patescibacteria are associated with the migration of metals in the soil, so representatives of this phylum were noted in fallow soils in mineral horizons, while in mature soil, they were practically not represented [47]. The formation of anaerobic conditions in soils of fallow lands determines the presence of representatives of the phylum Gemmatimonadota,

which are capable of anoxygenic photosynthesis in soil [48]. This group of bacteria has a wide range of metabolic capabilities, including the transformation of complex organic compounds into plant-available forms. They can act as rhizobacteria to stimulate plant growth and protect plants from pathogens. These phyla are practically absent in the native soil but are present in fallow soils, which may indicate active processes of organic matter transformation in the soil. The results of this study show that former agricultural use can disturb the microbiological stability of the environment, which is expressed in the change in the proportions between dominant oligotrophs and copiotrophs (Acidobacteriota, Gammaproteobacteria, Alphaproteobacteria, Verrucomicrobota). In comparison with native soil, this conclusion has been reached as well by other researchers [43,49].

Ecogenetic succession of phytocenoses in fallow lands leads to a significant change in the taxonomic composition of soil microbial communities. This is associated with the transition from herbaceous communities to trees, the formation of forest floors, and then the change from herbaceous communities to forest litter. Such transformations contributed to the transition from natural specialized microbial communities of natural forests to a diverse microbiome with a high proportion of copiotrophs. However, with increasing time of the transition to the fallow state, the content of organic matter, nutrients, and acidity decreases, which leads to the gradual restoration of the original oligotrophic composition of the microbial community. The greatest changes in the taxonomic composition of the soil microbiome were recorded in the upper old arable soil horizons, as well as in the forest litter and podzol horizons in the natural soil. Although morphologically, the changes were weakly expressed due to the weak transformation of arable horizons, the soil microbiome had differences among soils of the fallow lands of different ages. In the fallow soils, we could note the processes of forest litter formation in the native podzol, thus deepening the fallow-arable horizon (A_{hb}) and forming new horizons (O_e) at the boundary with fallow-arable horizons [42]. The transformation of fallow soils is strongly related to the time factor of soil formation [15]: with an increase in the time of the fallow state, we could observe changes in the taxonomic composition of the microbial community, as well as changes in the physico-chemical composition and morphological features of fallow soils. However, it is still an open question on how much time is needed for the formation of the natural microbiome in soils subjected to agricultural development and what processes may also contribute significantly to the change in the taxonomic composition of the microbial community.

3.3. Limitation of This Research

Very few mechanisms are known to explain the relationship between soil properties and soil biota at large spatial scales and for different land-use types, as most studies have traditionally focused on small spatial scales. This limitation prevents us from understanding broader patterns and trends in the composition and functioning of the soil microbiome. In addition, the complexity of the soil microbial community and its multiple roles in combination with various environmental parameters further hinder our ability to determine the interactions and impacts of the soil microbiome on ecosystem processes. In addition, the spatial heterogeneity in soil microbiomes presents a research challenge.

4. Conclusions

In this study, the evolutionary dynamics of the taxonomic composition of microbial communities in natural and different-aged soils were studied for the southern taiga sub-zone, and their association with physical and chemical parameters of soils, as well as the morphological organization of soils, were analyzed. It was found that the morphological organization of the different-aged fallow soils changed slightly over time. As a result of the transition to the fallow state, the accumulation of leached mineral grains was noted, indicating the process of leaching. As a result of the transition of soils to the fallow state, significant changes in the physicochemical parameters of soils were noted. With the increasing age of the fallow state, there was a decrease in the content of organic carbon in fallow-arable horizons, a decrease in the content of biogenic elements (P₂O₅, K₂O), and an increase in soil

acidity. Due to changes in the composition of plant communities, morphological organization of soils, and physico-chemical parameters, there was a change in the composition of the microbial community of fallow soils. According to the alpha diversity index, the richest microbiome was observed in the 40-year-old fallow soil; with increasing age, the alpha diversity index decreased, and the lowest alpha diversity index was observed in the native podzol. According to beta diversity analysis among the fallow and background soils, no clear pattern in the differences was revealed. According to the CCA analysis, it was found that the microbiome has a significant relationship with the content of exchangeable forms of biogenic elements, organic carbon, and soil acidity. The microbiome of fallow soils was characterized by a higher diversity of taxonomic composition, and the diversity decreased with increasing time in the fallow state.

Author Contributions: E.A. (Evgeny Abakumov), V.P., A.L. (Anton Lavrishchev) and A.L. (Andrey Litvinovich), conceptualization; E.A. (Evgeny Abakumov), funding and writing; V.P. and A.L. (Anton Lavrishchev), expedition with fieldwork and soil sampling; V.P., E.A. (Evgeny Abakumov), A.L. (Anton Lavrishchev) and A.L. (Andrey Litvinovich), wrote this paper; A.K., G.G. and E.A. (Evgeny Andronov), analysis of the soil microbiome. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Russian Scientific Foundation, in accordance with the agreement from 20.04.2023 No. 23-16-20003, and the Saint-Petersburg Scientific Foundation, in accordance with the agreement from 05.05.2023 No. 23-16-20003.

Data Availability Statement: Data are contained within the article.

Acknowledgments: This research was partially supported by scientific equipment of the Scientific Park of Saint-Petersburg State University, the Chemical Analysis and Materials Research Centre, and the Environmental Safety Observatory.

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

References

1. Poveda, J. Insect frass in the development of sustainable agriculture. A review. *Agron. Sustain. Dev.* **2021**, *41*, 5. [[CrossRef](#)]
2. Ray, P.; Lakshmanan, V.; Labbé, J.L.; Craven, K.D. Microbe to Microbiome: A Paradigm Shift in the Application of Microorganisms for Sustainable Agriculture. *Front. Microbiol.* **2020**, *11*, 622926. [[CrossRef](#)] [[PubMed](#)]
3. Santos, L.F.; Olivares, F.L. Plant microbiome structure and benefits for sustainable agriculture. *Curr. Plant Biol.* **2021**, *26*, 100198. [[CrossRef](#)]
4. Kughur, G.; Audu, O. Effects of Intensive Agricultural Production on the Environment in Benue State, Nigeria. *CAB Rev.* **2015**, *8*, 7–11.
5. Howard, M.M.; Kaminsky, L.M.; Kessler, A.; Bell, T.H. Merging microbial and plant profiling to understand the impact of human-generated extreme environments on natural and agricultural systems. In *Advanced Techniques for Studying Microorganisms in Extreme Environments*; Yergeau, É., Ed.; De Gruyter: Berlin, Germany; Boston, MA, USA, 2019; pp. 57–92.
6. Blundell, R.; Schmidt, J.E.; Igwe, A.; Cheung, A.L.; Vannette, R.L.; Gaudin, A.C.M.; Casteel, C.L. Organic management promotes natural pest control through altered plant resistance to insects. *Nat. Plants* **2020**, *6*, 483–491. [[CrossRef](#)] [[PubMed](#)]
7. Hartman, K.; van der Heijden, M.G.A.; Wittwer, R.A.; Banerjee, S.; Walser, J.-C.; Schlaeppi, K. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **2018**, *6*, 14. [[CrossRef](#)]
8. Hannula, S.E.; Zhu, F.; Heinen, R.; Bezemer, T.M. Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. *Nat. Commun.* **2019**, *10*, 1254. [[CrossRef](#)] [[PubMed](#)]
9. Jansson, J.K.; Hofmockel, K.S. Soil microbiomes and climate change. *Nat. Rev. Microbiol.* **2020**, *18*, 35–46. [[CrossRef](#)] [[PubMed](#)]
10. Kalinina, O.; Cherkinsky, A.; Chertov, O.; Goryachkin, S.; Kurganova, I.; Lopes de Gerenyu, V.; Lyuri, D.; Kuzyakov, Y.; Giani, L. Post-agricultural restoration: Implications for dynamics of soil organic matter pools. *Catena* **2019**, *181*, 104096. [[CrossRef](#)]
11. Lyuri, D.I.; Goryachkin, S.V.; Karavaeva, N.A.; Denisenko, E.A.; Nefedova, T.G. *Dynamics of Agricultural Lands of Russia in XX Century and Postagrogenic Restoration of Vegetation and Soils*; GEOS: Moscow, Russia, 2010.
12. Kalinina, O.; Goryachkin, S.V.; Karavaeva, N.A.; Lyuri, D.I.; Najdenko, L.; Giani, L. Self-restoration of post-agrogenic sandy soils in the southern Taiga of Russia: Soil development, nutrient status, and carbon dynamics. *Geoderma* **2009**, *152*, 35–42. [[CrossRef](#)]
13. Isachenko, G.A.; Isachenko, T.E. Approaches to cultural landscape zoning. *Proc. Russ. Geogr. Soc.* **2016**, *148*, 1–17.
14. Lyuri, D.I.; Karelin, D.V.; Kudikov, A.V.; Goryachkin, S.V. Changes in soil respiration in the course of the postagrogenic succession on sandy soils in the southern taiga zone. *Eurasian Soil Sci.* **2013**, *46*, 935–947. [[CrossRef](#)]
15. Abakumov, E. Rendzinas of the Russian Northwest: Diversity, Genesis, and Ecosystem Functions: A Review. *Geosciences* **2023**, *13*, 216. [[CrossRef](#)]

16. Sommer, R.; de Pauw, E. Organic carbon in soils of Central Asia—Status quo and potentials for sequestration. *Plant Soil* **2011**, *338*, 273–288. [CrossRef]
17. Giani, L.; Chertov, O.; Gebhardt, C.; Kalinina, O.; Nadporozhskaya, M.; Tolkdorf-Lienemann, E. Plagganthrepts in northwest Russia? Genesis, properties and classification. *Geoderma* **2004**, *121*, 113–122. [CrossRef]
18. Laganière, J.; Angers, D.A.; Paré, D. Carbon accumulation in agricultural soils after afforestation: A meta-analysis. *Glob. Chang. Biol.* **2010**, *16*, 439–453. [CrossRef]
19. Malakhovskiy, D.B. On the role of the latest tectonics in the relief formation of glacial areas of North-West Russia. *Izv. Russ. Geogr. Soc.* **2000**, *132*, 45–52.
20. Gagarina, E.I. *Lithological Factor of Soil Formation (by the Example of the North-West Russian Plain)*; Saint-Petersburg State University: Saint-Petersburg, Russia, 2004; p. 260.
21. Jahn, R.; Blume, H.P.; Spaargaren, O.; Schad, P. *Guidelines for Soil Description*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; p. 98.
22. World Reference Base for Soil Resources; Food and Agriculture Organization of the United Nations; IUSS Working Group WRB. *World Reference Base for Soil Resources 2014, Update 2015*; FAO: Rome, Italy, 2015; 195p.
23. Kroetsch, D.; Wang, C. Particle Size Distribution. In *Soil Sampling and Methods of Analysis*; Angers, D.A., Larney, F.J., Eds.; CRC Press: Boca Raton, FL, USA, 2008; pp. 713–725.
24. GOST. *Determination of Mobile Phosphorus and Potassium Compounds by Kirsanov Method Modified by CINAO*; Pryanishnikov Institute of Agrochemistry: Moscow, Russia, 2011; p. 8.
25. ISO/TS 14256-1-2003:2003; Soil Quality—Determination of Nitrate, Nitrite and Ammonium in Field Moist Soils by Extraction with Potassium Chloride Solution—Part 1: Manual Method. International Organization for Standardization: Geneva, Switzerland, 2003; p. 14.
26. Lane, D.J. 16S/23S rRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*; Stackebrandt, E., Goodfellow, M., Eds.; John Wiley and Sons: New York, NY, USA, 1991; pp. 115–175.
27. Muzyer, G.; de Waal, E.C.; Uitterlinden, A.G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695–700. [CrossRef] [PubMed]
28. Gladkov, G.; Kimeklis, A.; Zverev, A.; Pershina, E.; Ivanova, E.; Kichko, A.; Andronov, E.; Abakumov, E. Soil microbiome of the postmining areas in polar ecosystems in surroundings of Nadym, Western Siberia, Russia. *Open Agric.* **2019**, *4*, 684–696. [CrossRef]
29. Girden, E.R. *ANOVA: Repeated Measures*; Sage Publications, Inc.: Thousand Oaks, CA, USA, 1992.
30. Bates, S.T.; Berg-Lyons, D.; Caporaso, J.G.; Walters, W.A.; Knight, R.; Fierer, N. Examining the global distribution of dominant archaeal populations in soil. *ISME J.* **2011**, *5*, 908–917. [CrossRef]
31. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
32. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef]
33. McMurdie, P.J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **2013**, *8*, e61217. [CrossRef]
34. Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.; O'Hara, B.; Simpson, G.; Solymos, P.; Stevens, H.; Wagner, H. *Vegan: Community Ecology Package*, R Package Version 2.2-1. 2015, Volume 2, pp. 1–2. Available online: <https://cran.r-project.org/web/packages/vegan/index.html> (accessed on 20 December 2023).
35. Litvinovich, A.V. Postagrogenic Evolution of Well Cultivated Soddy-Podzolic Soils in the Northwestern Nonchernozemic Zone. *Agronomy* **2009**, *7*, 85–93.
36. Litvinovich, A.; Pavlova, O.; Lavrishchev, A.; Bure, V. Study of Indicators of Soil Fertility of Cultivated Sod-Podzolic Sandy Soil at Different Stages of Formation of Natural Ecosystems. *Agrochemistry* **2022**, *6*, 14–27.
37. Post, W.M.; Kwon, K.C. Soil carbon sequestration and land-use change: Processes and potential. *Glob. Chang. Biol.* **2000**, *6*, 317–327. [CrossRef]
38. Howard, M.M.; Kao-Kniffin, J.; Kessler, A. Shifts in plant–microbe interactions over community succession and their effects on plant resistance to herbivores. *New Phytol.* **2020**, *226*, 1144–1157. [CrossRef] [PubMed]
39. Barea, J.-M.; Pozo, M.J.; Azcón, R.; Azcón-Aguilar, C. Microbial co-operation in the rhizosphere. *J. Exp. Bot.* **2005**, *56*, 1761–1778. [CrossRef] [PubMed]
40. Li, Z.; Zheng, Y.; Li, Y.; Cheng, X.; Huang, S.; Yang, X.; Qin, Y. Genotype-Specific Recruitment of Rhizosphere Bacteria From Sandy Loam Soil for Growth Promotion of *Cucumis sativus* var. *hardwickii*. *Front. Microbiol.* **2022**, *13*, 910644. [CrossRef] [PubMed]
41. Orlova, O.V.; Kichko, A.A.; Chirak, E.L.; Zverev, A.O.; Lisina, T.O.; Andronov, E.E. Soil bacterial community during straw decomposition depending on the amount of available organic matter. *Eurasian Soil Sci.* **2023**, *5*, 626–639.
42. Choma, M.; Šamonil, P.; Kaštovská, E.; Bárta, J.; Tahovská, K.; Valtera, M.; Šantrůčková, H. Soil Microbiome Composition along the Natural Norway Spruce Forest Life Cycle. *Forests* **2021**, *12*, 410. [CrossRef]

43. Górska, E.B.; Stępień, W.; Hewelke, E.; Lata, J.-C.; Gworek, B.; Gozdowski, D.; Sas-Paszt, L.; Bazot, S.; Lisek, A.; Gradowski, M.; et al. Response of soil microbiota to various soil management practices in 100-year-old agriculture field and identification of potential bacterial ecological indicator. *Ecol. Indic.* **2024**, *158*, 111545. [[CrossRef](#)]
44. Wang, L.; Ye, X.; Hu, H.; Du, J.; Xi, Y.; Shen, Z.; Lin, J.; Chen, D. Soil bacterial communities triggered by organic matter inputs associates with a high-yielding pear production. *Soil* **2022**, *8*, 337–348. [[CrossRef](#)]
45. Lladó, S.; Žifčáková, L.; Větrovský, T.; Eichlerová, I.; Baldrian, P. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. *Biol. Fertil. Soils* **2016**, *52*, 251–260. [[CrossRef](#)]
46. Colette, M.; Guentas, L.; Patrona, L.D.; Ansquer, D.; Callac, N. Dynamic of active microbial diversity in rhizosphere sediments of halophytes used for bioremediation of earthen shrimp ponds. *Environ. Microbiome* **2023**, *18*, 58. [[CrossRef](#)] [[PubMed](#)]
47. Tian, Z.; Li, G.; Tang, W.; Zhu, Q.; Li, X.; Du, C.; Li, C.; Li, J.; Zhao, C.; Zhang, L. Role of *Sedum alfredii* and soil microbes in the remediation of ultra-high content heavy metals contaminated soil. *Agric. Ecosyst. Environ.* **2022**, *339*, 108090. [[CrossRef](#)]
48. Mujakić, I.; Piwosz, K.; Koblížek, M. Phylum Gemmatimonadota and Its Role in the Environment. *Microorganisms* **2022**, *10*, 151. [[CrossRef](#)]
49. Swędrzyńska, D.; Grześ, S. Microbiological Parameters of Soil under Sugar Beet as a Response to the Long-Term Application of Different Tillage Systems. *Pol. J. Environ. Stud.* **2015**, *24*, 285–294. [[CrossRef](#)]

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