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Posted Date: 25 February 2025

doi: 10.20944/preprints202502.1935.v1

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Assessment of Soil Health through Metagenomic Analysis of Microbiomes in Russian's Black Soil

Olesya O. Galanova [1], Nikita A. Mitkin [2], Albina A. Danilova [3], Vsevolod V. Pavshintsev [2], Denis A. Tsybizov [2], Alexander M. Zakharenko [3], Kirill S. Golokhvast [3,4], Tatiana V Grigoryeva [5], Maria I Markelova [5] and Aleksey A. Vatlin [2,*]

[1] Moscow Center for Advanced Studies, Moscow, Russia
[2] Institute of Ecology, Peoples' Friendship University of Russia (RUDN University), Moscow, Russia
[3] Siberian Federal Research Center of Agrobiotechnology RAS, Krasnoobsk, Russia
[4] Advanced Engineering School "Agrobiotek" of the Tomsk State University, Tomsk, Russia
[5] Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia
* Correspondence: vatlin_alexey123@mail.ru (A.A.V.)

Abstract: Soil health is a critical determinant of agricultural productivity and environmental sustainability. Traditional assessment methods often fail to provide a comprehensive understanding of soil microbial communities and their functions. This study addresses this challenge by employing metagenomic techniques to assess the functionality of soil microbiomes in Russian black soil, renowned for its high fertility. We utilized shotgun metagenomic sequencing to analyze soil samples from Western Siberia subjected to different degrees of agro-soil disturbance. We identified functional genes involved in carbon (*accA, argG, acsA, mphE, miaB*), phosphorus (*phoB, ppa, pstB, pnp, phnJ*), and nitrogen (*queC, amiF, pyrG, guaA, guaB, napA*) metabolic pathways and associated with changes in microbial diversity in general and higher representation of certain bacterial species - *Bradyrhizobium spp*. Results demonstrated significant differences in microbial composition and functional potential between tillage treatments. No-Till technology and conventional tillage practices promoted beneficial microbial communities and enhanced soil health compared to long-term fallow soil. This work underscores the potential of metagenomic analysis in providing a comprehensive understanding of soil health, marking a significant advancement in the field.

Keywords: soil metagenome; Russian black soil; shotgun sequencing, agricultural productivity; No-Till technology

1. Introduction

Soil health is a critical component of agricultural productivity, environmental sustainability, and overall ecosystem function. Healthy soils support plant growth by providing essential nutrients, water, and a stable structure for roots, while harboring beneficial microorganisms that enhance nutrient availability and protect plants from diseases. Soil health is linked to environmental sustainability through its role in carbon sequestration, water infiltration, and erosion control, which contribute to climate change mitigation and water quality protection [1,2]. Despite the importance of soil health, traditional assessment methods often fail to provide a comprehensive understanding of soil microbial communities and their functions [3]. Metagenomic analysis has emerged as a powerful tool for investigating soil health by characterizing microbial communities and their functional potential. This approach involves sequencing the collective genomes of microorganisms present in a soil sample, offering insights into the diversity, structure, and functional capabilities of the soil microbiome [4]. However, the high microbial diversity and variable evenness in soil pose challenges for metagenomic assembly, making it difficult to reconstruct microbial genomes from soil samples [5].

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Recent advances in metagenomic methods have allowed the characterisation of microbial indicators of soil health as influenced by different types of tillage. These techniques can assess both compositional and functional changes in microbial communities, providing valuable insights into soil health [6]. Field-scale studies investigating microbial taxa from agricultural experiments are crucial for understanding the long-term effects of crop rotation and tillage on microbial indicator species [7] and to identify bioindicators of soil health by characterizing the changes caused by tillage [8].

Our research centers on evaluating soil health through metagenomic analysis of the microbiomes found in Russian black soil. Renowned for its exceptional fertility and high organic matter content [9], Russian black soil provides a distinctive setting for investigating soil microbial communities. Nevertheless, the impact of various tillage practices on the microbial diversity and functional potential of this soil type has not been thoroughly explored. This study will enable the examination of soil samples from fields that have undergone different tillage methods: Long-term fallow (for 16 years) and two types of grain–fallow crop rotation: conventional tillage and No-Till technology [10].

We applied metagenomic sequencing using Illumina technology to assess the taxonomic diversity of soil microbiomes and to identify genes related to the cycles of phosphorus, nitrogen and carbon accumulation in soil. Our results showed significant differences in microbial composition between different tillage practices. No-Till technology and convetional tillage practices promoted beneficial microbial communities and soil health by improving nutrient cycling and organic matter decomposition. This paper describes a process for assessing soil health using metagenomic analysis of Russian chernozem microbiomes, demonstrating the potential of metagenomic methods in providing a comprehensive understanding of soil microbial communities and their functions. Our study highlights the significant impact of different tillage practices on soil health and the need for sustainable soil management practices to ensure long-term soil fertility and productivity.

2. Materials and Methods

1. Study area and collection of soil samples

Soil samples were collected in August 2023 from medium-humus, medium-loamy black soil (Luvic Chernozem) in the forest-steppe region of the Ob area (coordinates: 54° 53' 13.5" N, 82° 59' 36.7" E). The size of the experimental plots occupied by wheat was 15m x 18m. Soil samples were collected at the 0-10 and 10-20 cm soil layers. The sample for analysis was an average sample of 5 individual ones, collected at each spatial replication of the experiment. On the long term fallow, soil sampling was carried out according to the same scheme. Thus, 18 samples were obtained: 3 variants, 2 layers, 3 replicates. The soil samples were delivered to the laboratory, passed through a sieve with a cell diameter of 1 mm under sterile conditions, placed in sterile containers and stored at a temperature of minus 80 degrees until analysis.

2. Analysis of physical and chemical properties of soil samples

The total organic carbon (TOC) content was determined via the dichromate oxidation method [11]. The mortmass was separated by decanting the soil with water on a sieve with a cell diameter of 0.25 mm. The biomass was dried to an absolutely dry state. The carbon content was determined by thermal analysis on a Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany) according to manufacturer's protocols. The amount of microbial biomass was determined using a substrate-induced respiration method [12]. N-NO3 was determined by the ionometric method. Extractant 1 N K2SO4 solution at a soil:solution ratio of 1:2. The determination of nitrates was carried out using ion-selective electrodes [13]. Phosphatase activity in soil samples was determined using sodium phenolphthalein phosphate as a substrate (pH 6.5), incubation at 30 oC for 60 min [14].

3. DNA extraction:

DNA was extracted using the Quick-DNA Fungal/Bacterial Microprep Kit/Quick-DNA Fecal/Soil Microbe Microprep kit (Zymo Research) following the manufacturer's protocol. The extracted DNA was quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA,

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USA) and quality was assessed by electrophoresis in 1% agarose gel. Additionally, DNA quality was assessed according to the manufacturer's instructions from Agilent Technologies using the 2100 Bioanalyzer.

4. 'Shotgun' sequencing using Illumina technology.

DNA samples were used to create libraries using Illumina DNA Prep, (M) Tagmentation (100 ng). Shotgun sequencing using Illumina Novaseq technology was performed using the manufacturer's standard techniques. Q30 value: ≥93.00 % (Percentage of sequenced bases with a Phred score ≥30. This value is based on the entire sequencing run). Read length: 2x101 bp. The data was uploaded to the NCBI under BioProject: PRJNA1226397.

5. Primary bioinformatic data processing.

Demultiplexing of sequencing reads was performed using Illumina bcl2fastq (2.20). Adapters were trimmed using Skewer software (version 0.2.2) [15]. The quality of FASTQ files was analysed using FastQC software (version 0.11.5-cegat).

6. Bioinformatic analyses of taxonomic diversity

Taxonomic analysis was conducted using Kraken2 (v.2.1.13) [16] and relative abundance was calculated using Bracken (v.2.9) at the 'genus' and 'species' levels[17]. Alpha diversity was conducted using Shannon index calculating to establish the level of taxons' diversity within groups. Beta diversity was calculated using Bray-Curtis distance matrix and PERMANOVA analysis to identify taxons' differences between groups. The vegan, adonis and edgeR packages were used [18,19]. To establish differences in relative abundance among different sample groups of the soil microbiome, the U-criterion (Mann-Whitney test) and false positive rate (FDR) by the Benjamini–Hochberg method were calculated. The stats, matplotlib, numpy and pandas libraries were used (Available online: https://matplotlib.org/stable/index.html, https://numpy.org/doc/, https://api.semanticscholar.org/CorpusID:61539023).

7. Functional analysis

To perform a functional analysis, it was necessary to collect the catalog of target amino acid sequences. To do this, we have compiled the list of target carbon, nitrogen, and phosphorus exchange genes and taxa that we obtained at the stage of taxonomic analysis. Using this data, we conducted multiple searches in the UniProt [20] and KEGG [21] database. The catalog was compiled from the detected sequences. All sequences satisfy the following requirements: i) the amino acid sequence belongs to the carbon, phosphorus or nitrogen metabolic pathway; ii) the amino acid sequence belongs to an organism that was obtained in the results of the taxonomic analysis (up to class level) iii) the sequence must have at least 2 annotations in the UniProt database. Full catalog information can be viewed in Table S1.

Target genes from the compiled catalog were identified within the sequencing reads using blastx Diamond (v.2.1.11) [22]. To interpret the results, we conducted the following requirements: i) the alignment must have at least 90% homology; ii) the alignment length corresponding to the length of the read. The readcounts were calculated for the detected coding sequences of genes. The calculation was performed using our own code. Then readcounts were normalized using the Trimmed Mean of M-values (TMM) normalization method. To identify significant differences in gene abundance between the groups, the Mann-Whitney test and FDR by the Benjamini–Hochberg method were calculated. If FDR < 0.05, we assume that the abundance of genes in the groups is statistically significantly different in comparing groups.

The results of the taxonomic and functional analysis were compared into a signature matrix consisting of pairs (taxon; gene). Matrix data was processed with TMM normalization, the U-criterion and FDR using the Benjamini–Hochberg method.

3. Results

3.1. Assessment of Soil Physico-Chemical Parameters

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The central forest-steppe of the Novosibirsk Priobye, where soil samples were taken, belongs to the eastern edge of the Priob plateau and is located at the junction of two major geomorphological provinces of the West Siberian Plain and the mountain systems of the Kuznetsk Ala-Tau, Altai and Sayan. Absolute heights of the Priobie region are 200-250 metres above sea level. According to the agroclimatic zoning of the Novosibirsk Oblast, the central forest-steppe of the Priobie region belongs to the moderately warm, moderately humidified agroclimatic sub-area (Agroclimatic Resources of the Novosibirsk Oblast, 1971). The difference between the average monthly temperatures of the hottest month and the coldest month is 38°C. Winter is cold and long. Stable snow cover is retained for 157-162 days. Soils freeze up to 1.8-2 meters.

Leached black earths (Luvic Chernozem) are the most fertile soils of Western Siberia. The main crops of grain crops are located on these soils. Since the mid-1980s of the 20th century, large-scale research has been carried out to develop soil-protective farming in the forest-steppe zone of Western Siberia [23]. In particular, it is shown that redistribution of C in mortmass in the profile of root-bearing layer at minimization of mechanical tillage is the main reason of change of its living phase [24].

Long term permanent fallows are an informative tool for studying the problems of soil system stability under conditions of agrogenic degradation [25,26]. In our experiment, the soil was without plants for 16 years, annual plowing was carried out in the spring and during the growing season weeds were removed with a cultivator. Conventional moldboard plowing to a depth of 25 cm is a generally accepted technology for cultivating soil for grain crops in Siberia. No-Till technology is currently being implemented in Siberia

The selected series of options represents 3 degrees of disturbance of the agro-soil. Long-term fallow is a model of extreme degradation of agro-soil, in conditions of No-Till technology, when the intensity of mechanical impact is reduced, soil properties change towards enrichment of mobile fractions of organic matter due to redistribution and some conservation of plant residues, while conventional tillage is a kind of standard to average soil samples found in a given region. Previous research has demonstrated that various tillage methods significantly impact soil quality, carbon and nitrogen stocks, and soil aggregates [27,28].

Our physico-chemical analysis of the soil samples focused on phosphorus, carbon, and nitrogen content. The granulometric composition of the soil is medium loamy, coarse silt-sandy, the content of physical clay (particles <0.01 mm) is 38.3%, coarse silt (0.05-0.01 mm) - 25.0%, fine sand (0.25-0.05 mm) - 36.2%. All samples have pH=7. The main physical and chemical properties of the soil are presented in Table 1

Table 1. Properties of leached medium-humus, medium-loamy chernozem (Luvic Chernozem) of the Ob forest-steppe of different types of treatment.

Soil indicator	Long-term fallow	Conventional tillage	No-Till technology
TOC %	4.1	4.2	
C in mortmass, mg/kg	67	817	1133
C in microbial biomass, μg/g	30	100	120
N-NO3, mg/kg	78.6	19.8	4.4
Phosphatase activity, μg, P2O5/g per hour	13.8	14.6	26.2

3.2.2. Taxonomy Analysis

The alpha diversity analysis revealed a high level of species diversity, with no statistically significant differences observed among subgroups subjected to varying tillage practices. The Shannon diversity index, averaging approximately 7.7 across all groups, indicates a notably high level of species richness and evenness [29]. This suggests a substantial heterogeneity in species composition within each soil subgroup. Such elevated biodiversity is essential for sustaining soil

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health and fertility, as it plays a pivotal role in regulating nutrient cycling and the decomposition of organic matter (Figure 1).

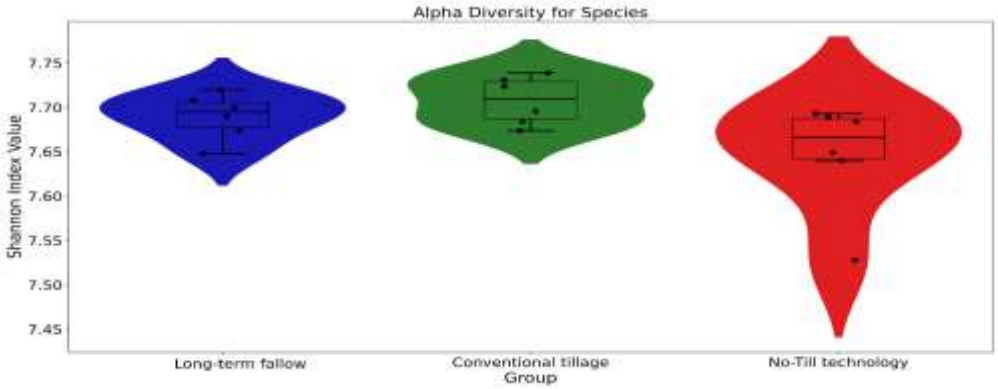

Figure 1. Alpha diversity of the taxonomic composition of Leached black earths. The values of the Shannon index are located on the Y-axis. On the X-axis, there are subgroups of soil samples. The shape of the graph represents the distribution density of the Shannon index. The boxplot shows the median, quartiles, and spread of the Shannon index among the data.

The beta diversity analysis, conducted using the Bray-Curtis dissimilarity matrix and PERMANOVA, revealed statistically significant differences among the subgroups. The NMDS graph (Figure 2) further corroborates these findings, demonstrating clear clustering of samples into three distinct subgroups corresponding to the tillage practices. At the same time, the data exhibited considerable dispersion at the species level. The PERMANOVA results indicated that approximately 40% of the observed variability in the data set can be attributed to the classification of soil samples into the three tillage practice groups.

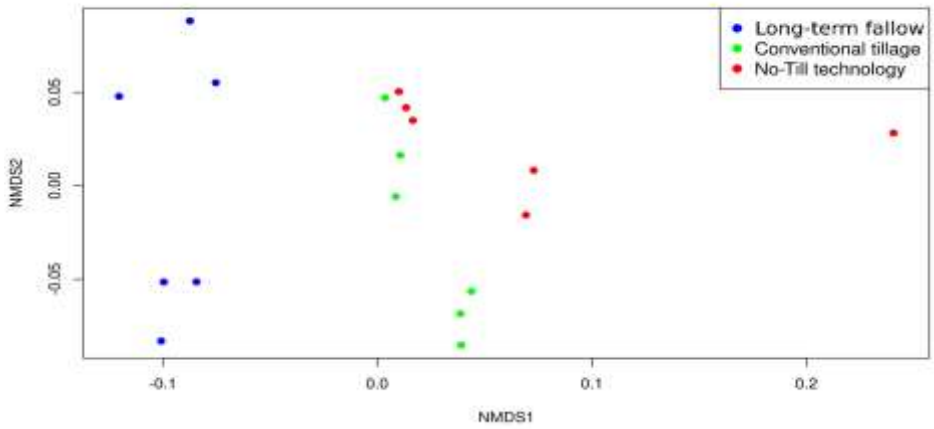

Figure 2. Beta diversity of the taxonomic composition of Leached black earths at the level of species. The NMDS graph shows the clustering of soil samples (dots on the figure). In our case, the samples are clearly divided into 3 subgroups, the results of the PERMANOVA test indicate that this result is statistically significant (p-value = 0.01). The blue dots represent soil samples from samples subgroup with long-term fallow, green dots for soil samples with conventional tillage and red dots for soil samples with No-Till technology.

The relative representation of taxa in groups was also compared using the Wilcoxon-Mann-Whitney test (U-test) and FDR. The graph (Figure 3) shows statistically significant results, with median values for each group.

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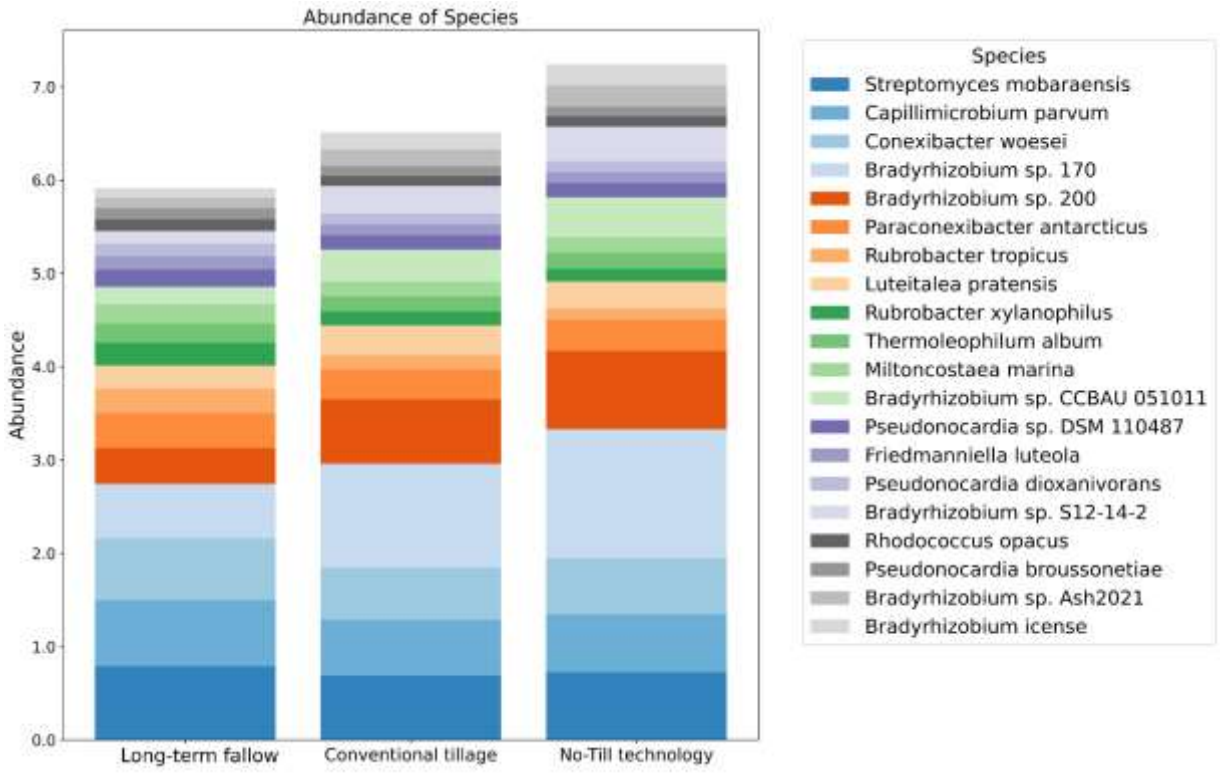

Figure 3. Comparison of median values of relative representation of species for groups of leached black earths. The relative representation of species in % is located on the Y-axis. On the X-axis, there are subgroups of soil samples.

At the species level, the most obvious difference is in the representation of Streptomyces mobaraensis, Capillimicrobium parvum, Conexibacter woesei, Bradyrhizobium sp. 170, Bradyrhizobium sp. 200, Bradyrhizobium sp. CCBAU 051011, Bradyrhizobium sp. S12-14-2. The differences in the representation of these species are more than 0.5%.

The abundance of species *Streptomyces mobaraensis*, *Capillimicrobium parvum* and *Conexibacter woesei* is higher for soil samples with Long-term fallow in comparison with the conventional tillage and No-Till technology groups.

The species *Bradyrhizobium sp.* 170 is the most represented in subgroup of No-Till technology and the least represented in the Long-term fallow group, as well as the species *Bradyrhizobium sp.* 200, *Bradyrhizobium sp.* CCBAU 051011, *Bradyrhizobium sp.* S12-14-2, and *Bradyrhizobium license*.

3.3. Analysis of Genes

The genes of carbon, nitrogen and phosphorus metabolism were searched using the catalog of homologues and reads. Relative abundances for the putative genes encoding enzymes were counted and compared, as described in the 'Materials and Methods' section. The genes with statistically significant difference in relative abundance between soil subgroups are presented in Figure 4.

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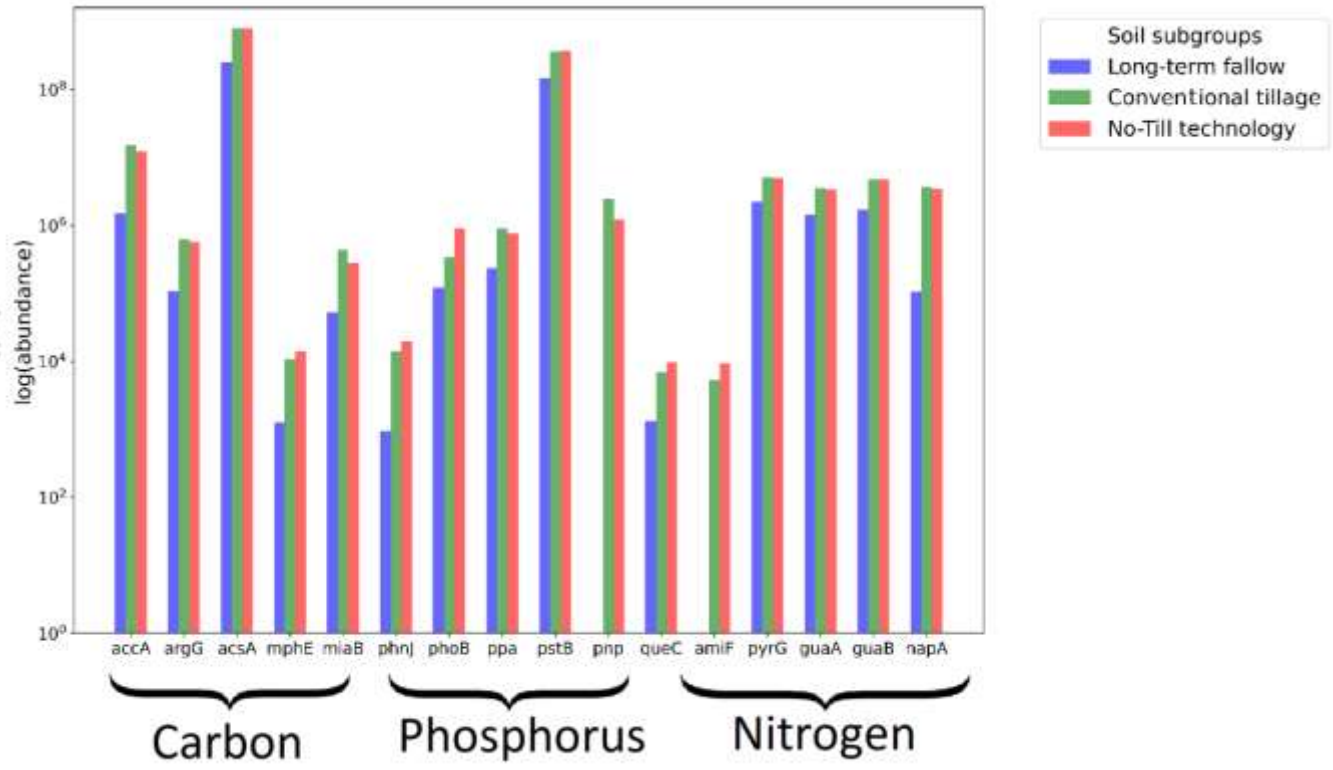

Figure 4. Comparison of median values of relative representation of species for groups of leached black earths. The relative representation of species in % is located on the Y-axis. On the X-axis, there are subgroups of soil samples.

The relative abundance of the genes from Figure 4 is presented as median relative abundance of the gene in soil samples subgroups (Appendix A, Table A1). The annotation (function and belonging to the metabolic pathway) to these genes is performed in Table 2.

Table 2. Annotation for genes with statistically significant differences in relative abundance about their function and belonging to the metabolic pathway (carbon, nitrogen, phosphorus).

Gene name	Enzyme name	KEGG enzyme entry	Metabolic pathway
accA	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	2.1.3.15	carbon
argG	Argininosuccinate synthase (Forming carbon-nitrogen bonds)	6.3.4.5	
acsA	Acetyl-coenzyme A synthetase	6.2.1.1	
mphE	4-hydroxy-2-oxovalerate aldolase	4.1.3.39	
miaB	tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase	2.8.4.3	

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	(catalyzes methylation)		
phnJ	Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P lyase	4.7.1.1	phosphorus
phoB	Phosphate regulon transcriptional regulatory protein	3.6.1.11	
ppa	Inorganic pyrophosphatase	3.6.1.1	
pstB	Phosphate import ATP-binding protein	7.3.2.1	
pnp	Polyribonucleotide nucleotidyltransferase (catalyzes the phosphorolysis)	2.7.7.8	
queC	7-cyano-7-deazaguanine synthase (Forming carbon-nitrogen bonds)	6.3.4.20	nitrogen
amiF	Formamidase (Acting on carbon-nitrogen bonds)	3.5.1.49	
pyrG	CTP synthase (glutamine hydrolysing) (Forming carbon-nitrogen bonds)	6.3.4.2	
guaA	GMP synthase [glutamine-hydrolyzing] (Forming carbon-nitrogen bonds)	6.3.5.2	
guaB	Inosine-5'-monophosphate dehydrogenase (Acting on the CH-OH group of donors)	1.1.1.205	
napA	Periplasmic nitrate reductase	1.9.6.1	

The relative abundance of genes differs significantly for soil subgroup 'Long-term fallow' and 'Conventional tillage', 'Long-term fallow' and 'No-Till technology'. The difference between 'Conventional tillage' and 'No-Till technology' is not statistically significant. In all cases, the relative abundance of genes for 'Long-term fallow' is significantly lower than for the other subgroups. Genes *acsA, accA, pstB* are the most represented (much more than 10^6). Genes *amiF* and *pnp* are not represented in 'Long-term fallow' (the median value of the readcounts is zero). Genes *mphE, phnJ* and *queC* are presented in 'Long-term fallow' at a significantly lower level compared to other subgroups and genes (lower than 10^4).

3.3. Signature Analysis

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To provide the results, all pairs containing the species discussed in the 'Taxonomy analysis' section (the species with the largest difference in representation between the subgroups) were selected. These species are *Streptomyces mobaraensis*, *Capillimicrobium parvum*, *Conexibacter woesei*, *Bradyrhizobium sp.* 170, *Bradyrhizobium sp.* 200, *Bradyrhizobium sp.* CCBAU 051011, *Bradyrhizobium sp.* S12-14-2. The Mann-Whitney test and FDR by the Benjamini–Hochberg method was calculated for the pairs. Then a heatmap was built to visualize the differences in their median relative abundance (Figure 5).

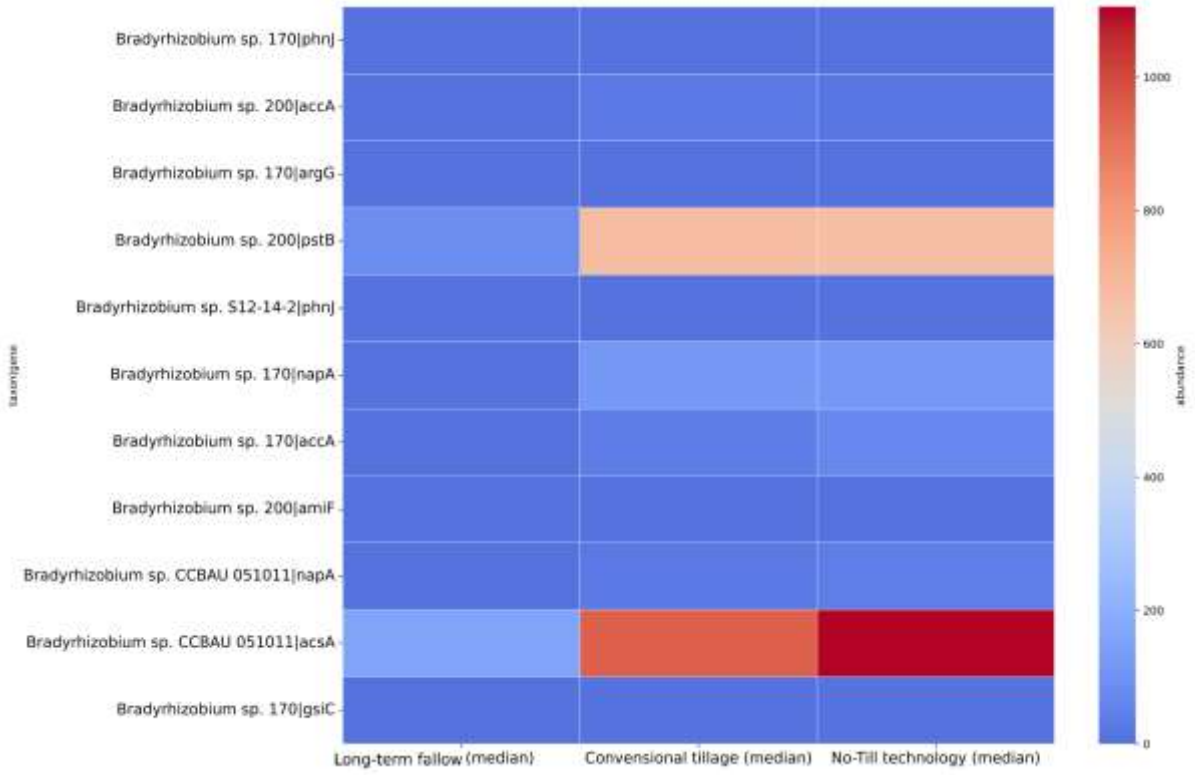

Figure 5. The heatmap for signature pairs, compiled according to their median relative abundance (FDR < 0.05). The center of the scale is set at 500 readcounts. Here we can see, that the highest abundance is for '*Bradyrhizobium sp.* CCBAU 051011|acsA' and '*Bradyrhizobium sp.* 200|pstB'

The heatmap shows that the signature pair '*Bradyrhizobium sp.* CCBAU 051011|acsA' has the highest abundance for 'No-Till technology' (over 1000 readcounts in median) and 'Conventional tillage' (about 1000 readcounts in median) in comparison with 'Long-term fallow'. The signature pair '*Bradyrhizobium sp.* 200|pstB' has much more high abundance (about 700 readcounts) in tilled groups compared with 'Long-term fallow' subgroup. These results support the results of taxonomic and functional analysis.

4. Discussion

Modern agriculture faces a number of challenges such as soil degradation, loss of fertility and the need for sustainable agricultural practices. Integrated soil health assessment methods, particularly through metagenomic analysis, can help to address these challenges. Our study contributes to this growing body of knowledge by employing metagenomic tools to evaluate the microbial communities and functional potential of Russian black soil (chernozem), a soil type renowned for its high fertility and agricultural importance [9]. Despite its inherent fertility, chernozem is not immune to degradation, and its sustainable management is critical to preserving its productivity [30]. By employing metagenomic techniques, we have been able to provide a comprehensive understanding of the microbial communities inhabiting this soil type and their

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functional potential. Using advanced metagenomic methods, we have gained insight into microbial diversity and genes that may be critical for maintaining soil health and productivity.

Our key findings highlight significant differences in microbial community composition nder different tillage regimes. No-Till technology and conventional tillage practices both promoted beneficial microbial communities (Figures 1, 2, 3) that potentially enhanced soil health by improving nutrient cycling and organic matter decomposition comparing to long-term bare fallow soil. These findings correspond to the increase in total carbon in microbial biomass (Table 1) and are consistent with previous studies demonstrating the positive impact of permanent tillage on soil microbial diversity and community stability [31]. At the same time, No-Till technology, which is considered a reduced tillage practice [32], was associated with a more heterogeneous soil environment than in case of conventional tillage, providing diverse ecological niches that support a wider range of microbial lifestyles. This diversity is crucial for maintaining the resilience of soil ecosystems and their ability to provide essential services, such as nutrient availability and disease suppression, to plants and their environment [33]. The increased microbial diversity under reduced tillage conditions is likely due to the preservation of soil structure and organic matter, which provides a stable habitat for microbial communities. In contrast, conventional tillage disrupts soil aggregates, leading to the loss of organic matter resulted in less extensive microbial diversity [34]. In general, both tillage methods positively influenced soil microbial diversity, with reduced tillage No-Till technology demonstrating greater effectiveness.

The relative representation of the major soil species, *Streptomyces mobaraensis*, *Capillimicrobium parvum* and *Conexibacter woesei*, inversely correlates with the increase in total microbial diversity (Figure 3). *S. mobaraensis* and *C. woesei* are known to form extended hyphal networks, and their abundance is associated with stable, structured soils where they thrive in protective microhabitats within intact soil aggregates [35,36]. *C. parvum*, a small-sized actinobacterium, is likely sensitive to rapid changes in soil moisture and organic matter distribution, which occur while applying regular tillage procedures [37]. Both tillage practices that break up soil aggregates can expose the described major bacterial species to harsher conditions—reducing their capacity to colonize and persist in the soil matrix, but at the same time, enabling the growth and development of other bacterial species.

The results of the study indicate that both treatment technologies led to a significant increase in the abundance of a certain microbial taxa: the members of the genus Bradyrhizobium. *Bradyrhizobium spp.* are well-known symbiotic nitrogen-fixing bacteria that form nodules on the roots of leguminous plants [38] and are responsible for heavy metal resistance and bioremediation in a long-term heavy metal-contaminated ecosystem[39]. *Bradyrhizobium* spp. have been shown to stimulate plant growth and improve plant resistance to biotic and abiotic stresses, further highlighting their importance as indicators of soil health and plant vitality [40,41]. Extended presence of *Bradyrhizobium* spp. under reduced tillage conditions (No-Till technology) suggests that these practice not only enhances soil fertility but also contribute to the resilience of soil ecosystems in the face of environmental stressors.

The functional analysis (Figure 4) revealed that genes associated with carbon metabolism, such as accA, argG, acsA, mphE, and miaB, were more abundant in soils subjected to No-Till technology and conventional tillage practices. Among them, accA (acetyl-coenzyme A carboxylase) and acsA (acetyl-coenzyme A synthetase) are especially crucial for carbon fixation and the synthesis of acetyl-CoA, a key intermediate in the carbon cycle [42]. The increased abundance of these genes due to the application of tillage suggests enhanced carbon sequestration and organic matter decomposition, which are critical for maintaining soil fertility [34]. This assumption is supported by the fact that both methods of soil treatment led to significantly higher carbon content in microbial biomass and the mortmass, as well as increased CO2 emission, comparing to an untreated soil (Table 1). Signature analysis (FIgure 5) identified *Bradyrhizobium sp.* CCBAU 051011 as a crucial bacterial species associated with the increased abundance of acsA in tilled soils, highlighting its significant role in carbon fixation processes.

The optimization of carbon and nitrogen metabolism in soils under agricultural practices has been extensively studied in the literature. However, significantly less attention has been given to the

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dynamics of phosphorus metabolism [43]. In particular, the influence of soil cultivation methods on phosphorus metabolism remains underexplored. Nonetheless, a global meta-analysis of 5876 observations demonstrated that soil conservation practices significantly enhance soil phosphatase activity [44]. Our findings provide additional support for this conclusion through the lens of soil metagenomic analysis. Phosphorus metabolism was significantly affected by tillage practices, with genes such as *phoB, ppa, pstB, pnp* and *phnJ* exhibiting higher abundance in soils subjected to treatment. These genes play essential roles in phosphorus uptake and regulation, processes vital for sustaining soil phosphorus availability and supporting plant nutrition [45]. Specifically, *phoB, ppa, phnJ* and *pstB* encode key proteins involved in solubilizing soil phosphate, thereby enhancing its accessibility to plants [46]. For instance, *phoB* is crucial for the regulation of phosphate uptake and solubilization as well as P-starvation response, since it controls the expression of phosphatases and phosphate transporters [47]. In tilled soils, the higher abundance of *phoB* suggests an adaptive response to phosphorus scarcity, promoting the solubilization of inorganic phosphate and enhancing its availability to plants. The increase of *phoB* abundance was 3 times on plowing and 7 times on No-Till compared to fallow, which may be a sign of optimization of phosphorus metabolism conditions under soil conservation technology. Higher presence of *ppa*, encoding an inorganic pyrophosphatase, corresponds to elevated phosphatase activity (Table 1) in tilled soils that is potentially associated with enhanced mineralization of organic phosphorus compounds, releasing orthophosphate for plant uptake [48]. The abundance of *phnJ*, a gene associated with the mineralization of organic phosphorus compounds [49], increased by two orders of magnitude compared to fallow conditions. The abundance of *pstB* gene, encoding a phosphate transporter protein involved in phosphorus uptake and transport, was 2.5 times higher in tilled soils than on fallow. Signature analysis (Figure 5) further highlighted that the elevated presence of *pstB* in tilled soils is linked to the increased abundance of *Bradyrhizobium sp.* 200, suggesting this species plays a significant role in phosphorus solubilization.

In the context of nitrogen metabolism, genes such as *queC, amiF, pyrG, guaA, guaB, napA* were more abundant in soils subjected to tillage practices. These genes are essential for nitrogen fixation and nitrate reduction, processes that are critical for soil fertility and plant growth. The increased abundance of these genes under tillage conditions is consistent with the higher levels of microbial biomass and the significant reduction in nitrate-nitrogen (N-NO3) soil content (Table 1). N-NO3 reduction is likely due to the enhanced activity of microbial communities carrying nitrogen metabolism genes, which promotes the immobilization of nitrogen in organic forms and reduces nitrate leaching [50]. Nitrate leaching is a major environmental concern, as it can lead to the contamination of groundwater and surface water bodies, contributing to eutrophication and other ecological imbalances [51]. By minimizing nitrate losses, tillage practices not only improve soil health but also contribute to broader environmental sustainability. High presence of nitrogen metabolism genes in tilled soils comes to an agreement with increased abundance of *Bradyrhizobium* spp., renowned for their ability to fix free nitrogen through symbiotic relationships with legumes and reported to carry the genes of this cluster [52].

5. Conclusions

This study demonstrates that different tillage practices significantly influence the functional genes and microbial taxa associated with key biogeochemical cycles in Russian black soil. No-tillage technology and conventional tillage practices promote beneficial microbial communities and enhance soil health by improving nutrient cycling and organic matter decomposition. The increased abundance of genes involved in carbon, nitrogen, and phosphorus metabolism, as well as the microbial taxa responsible for these processes, underscores the importance of adopting sustainable tillage practices to maintain soil fertility and productivity. The use of metagenomic analysis provides a comprehensive understanding of soil microbial communities and their functions, demonstrating high correlation with traditional widely-used physical and chemical methods evaluating soil quality/fertility and offering valuable insights for the development of sustainable agricultural practices.

 doi:10.20944/preprints202502.1935.v1

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Catalog information;

Author Contributions: Conceptualization, O.O.G., N.A.M., V.V.P., D.A.T. and A.A.V.; methodology, A.A.D., T.V.G. and M.I.M.; software, O.O.G.; formal analysis, O.O.G.; investigation, K.S.G.; resources, A.M.Z.; writing—original draft preparation, O.O.G., N.A.M. and A.A.V..; writing—review and editing, O.O.G., N.A.M. and A.A.V..; visualization, O.O.G., N.A.M. and A.A.V..; supervision,K.S.G.; project administration, K.S.G.; funding acquisition, A.A.V. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Olesya O. Galanova[1], Nikita A. Mitkin[2], Albina A. Danilova[3], Vsevolod V. Pavshintsev[2], Denis A. Tsybizov[2], Alexander M. Zakharenko[3], Kirill S. Golokhvast[3,4], Tatiana V Grigoryeva[5], Maria I Markelova[5], Aleksey A. Vatlin

Funding: This work was supported by the RUDN University Scientific Projects Grant System, project № 202760-2-000

Acknowledgments: We express gratitude to the Association of Specialists in the Field of Molecular, Cellular and Synthetic Biology (Russia) for their efforts in uniting specialists involved in this study.

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

Appendix A

Table A1. Median relative abundances for genes with statistically significant differences.

Gene	Long-term fallow	Conventional tillage (median)	No-Till technology (median)
accA	$1.53 \cdot 10^{6}$	$15.15 \cdot 10^{6}$	$12.48 \cdot 10^{6}$
acsA	$2.55 \cdot 10^{8}$	$8.09 \cdot 10^{8}$	$8.08 \cdot 10^{8}$
amiF	0	$5.38 \cdot 10^{3}$	$9.43 \cdot 10^{3}$
argG	$108.81 \cdot 10^{3}$	$624.34 \cdot 10^{3}$	$578.59 \cdot 10^{3}$
guaA	$1.44 \cdot 10^{6}$	$3.54 \cdot 10^{6}$	$3.41 \cdot 10^{6}$
guaB	$1.73 \cdot 10^{6}$	$4.79 \cdot 10^{6}$	$4.72 \cdot 10^{6}$
miaB	$53.03 \cdot 10^{3}$	$441.45 \cdot 10^{3}$	$281.52 \cdot 10^{3}$
mphE	$1.27 \cdot 10^{3}$	$10.87 \cdot 10^{3}$	$14.29 \cdot 10^{3}$
napA	$106.36 \cdot 10^{3}$	$3.67 \cdot 10^{6}$	$3.42 \cdot 10^{6}$
phnJ	$0.94 \cdot 10^{3}$	$14.27 \cdot 10^{3}$	$20.07 \cdot 10^{3}$
phoB	$121.40 \cdot 10^{3}$	$343.04 \cdot 10^{3}$	$913.79 \cdot 10^{3}$
pnp	0	$2.46 \cdot 10^{6}$	$1.22 \cdot 10^{6}$
ppa	$236.70 \cdot 10^{3}$	$910.72 \cdot 10^{3}$	$772.52 \cdot 10^{3}$
pstB	$1.49 \cdot 10^{8}$	$3.68 \cdot 10^{8}$	$3.72 \cdot 10^{8}$
pyrG	$2.22 \cdot 10^{6}$	$5.18 \cdot 10^{6}$	$4.96 \cdot 10^{6}$
queC	$1.32 \cdot 10^{3}$	$6.84 \cdot 10^{3}$	$9.72 \cdot 10^{3}$

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