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Research Article

Identification of Microbial Populations Present in Agricultural and Nonarable Soils in the Talas Valley, Northern Kyrgyzstan, in Autumn

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Soil bacterial and fungal communities were investigated in relation to soil type and farm management practices after vegetation harvesting in autumn. Soils from fields cultivated with *Phaseolus vulgaris* (bean) and *Pyrus comminus* (pear) and nonarable, natural areas were studied. Microbial diversity was analysed using cultivation-dependent methods (isolation of pure cultures) and cultivation-independent methods (direct extraction of DNA from soil, followed by PCR amplification of the 16S rRNA and 18S rRNA genes). The use of cultivation-dependent methods revealed that there were no differences in the biodiversity of the soil bacterial and fungal communities between fields cultivated with bean plants and pear trees. However, the use of cultivation-independent methods showed that there were clear soil and crop type-specific effects on the composition of the soil bacterial and fungal communities. The density of the bacterial population was two times higher in northern mountain-valley serozem (NMVS) soil samples than in light chestnut (LC) soil samples. In contrast, the densities of the fungal communities were almost equal in the studied soil types. The density of the actinomycetes community was almost two times higher in LC soil than in NMVS soil under bean plants. The Shannon index values showed that the bacterial biodiversity in the NMVS soil samples was greater than that in the LC soil samples. Soils under fallow appeared to have diverse bacterial communities that mainly consisted of local autochthonous microflora and a small amount of zymogenic microflora (since fresh plant residue does not enter the soil). The Shannon index results revealed two interesting facts: (1) the soil bacterial community was highly diverse in soils that supported bean plants and (2) the soil fungal biodiversity was high under pear trees in both soil types.

1. Introduction

Intensive farming with an annual application of fertilizers, the use of plant-protection chemicals, and various types of tillage and erosion all cause losses of the total soil carbon reserve, leading to an increase in the release of gases into the atmosphere, namely, N_2O and CO_2 [1–7].

Interestingly, almost 95% of the total soil biomass consists of microorganisms, and they play essential roles in soil formation, converting nutrients in nature from plant litter, maintaining the entire terrestrial ecosystem, and protecting the environment [8]. Hence, microorganisms are an indispensable part of living soil and essential for soil health. The soil microbial communities are diverse, and their diversity and abundance are primarily influenced by altitude and ecological and geological factors, such as the vegetation type, temperature, and soil pH [9–11]. Climate is another factor that directly affects soil microorganisms, and global warming has been identified as a potential environmental problem in Central Asia, particularly in Kyrgyzstan [12].

While some studies have revealed that soil type affects the microbial community structure far more than plant species, it is known that the exudates of various plants differ significantly in chemical composition [13, 14]. Others have also observed that both soil and plants could affect the community structure of soil microbes [15–19].

The level of microbial biodiversity in the soil determines the functioning of the ecosystem; for example, a decrease in

species diversity can affect the processes that control the nitrogen and carbon cycles. It is also worth noting that the composition of soil bacterial and fungal communities is dynamic, subject to strong fluctuations within short periods (e.g., months) during the growing season, and undergoes temporary changes that are dependent on tillage and crop rotation [20–22].

It has been reported that the rooting depth of perennial crops and the composition of the litter create a sensitive and dynamic microbial system that cycles plant residue [23]. Any change in the soil microbial biomass is regarded as an early indicator of change in the organic carbon cycle and soil organic matter [24, 25]. Studies have shown that the greater the biodiversity of soil microbes, the larger the plant biomass; that is, there is evidence of a relationship between the richness of bacteria and the stability of the plant biomass, especially the richness of fungi for plant biomass production [26]. Crop types (i.e., annual vs. perennial) and farming practices (i.e., the addition of fertilizers and manures) have also been shown to impact the dynamics of the soil microbial community and its biomass [27–31]. Plant species in long-term cultivation are the dominant driving force in the composition of soil microbial diversity [32–34]. Other studies have indicated that continuous cultivation of different agricultural plants has different effects on bacterial communities in different soil complexes (e.g., the rhizosphere soil and bulk soil) [35–37]. In addition, long-term use of high doses of nitrogen, phosphorus, and potassium (NPK) fertilizers reduces the richness of soil bacterial species [38, 39].

The aim of this study was to gain a better understanding of how land management, long-term cultivation, plant species, and soil types affect the soil microbial community and its biomass in the autumn after a harvest. More specifically, the main goal was to identify and compare the microbial communities present in long-term cultivated soils under two different plants and in uncultivated soils in the Talas region, North Kyrgyzstan.

The findings will support our understanding of the impact of land use on soil microbial communities and will be useful for predicting the future cycling of carbon and other nutrients in the region.

2. Materials and Methods

2.1. Short Description of the Study Area. The Talas region is located in northwest Kyrgyzstan. The area has a mid-temperate continental climate, with warm summers and cold winters. The annual average temperature is 6–8°C. The yearly precipitation is about 320 mm, and it generally occurs during the crop-growing season (late spring). The region is considered favourable for cultivating industrial crops; in recent years, farmers have grown beans in a monocultural manner and have obtained high yields. Commercial varieties of dwarf apple and pear trees are also cultivated. The beans and fruit trees are the most representative economic crops of this region; the seeds, fresh fruits, and products of these crops are exported to European countries.

2.2. Sampling Sites. The soil microbial communities present in natural and crop areas in the Talas region were analysed in autumn, after harvest in the crop areas, and after the growth of natural vegetation in the noncrop areas (Figures 1 and 2).

Nonarable soil samples, including soil samples under bean and pear trees, were collected in mid-September. The underground and aboveground organs of beans were still semidecomposing in the soil; pear trees still had green leaves, but the litter of yellow leaves predominated under the crown. Fluctuations in night and day temperatures were very noticeable; daytime air temperatures ranged from 15.0 to 19.00°C during the sampling period. Soil temperature ranged from 7.9 to 15.0°C (Table 1).

2.2.1. Characteristics of the Studied Soil Types. According to Mamytov [40, 41], the Talas region contains three types of soil: northern mountain-valley serozem (NMVS; low carbonate) soil, light chestnut (LC) soil, and dark chestnut soil. Cultivation has been practiced in some parts of the region for many years; however, other parts continue to support natural vegetation while being used for agricultural purposes, and livestock graze here during the spring and summer months.

(1) Northern Mountain-Valley Serozem (NMVS) Soil. NMVS soil is confined to the middle and lower parts of the Kyrgyz and Talas ridges' foothill plume within the absolute heights of 600–900 m. The mechanical composition of NMVS soil varies from skeletal sandy to cartilaginous silt loam. The humus content in the upper horizons fluctuates between 1.5 and 2.5%. The nitrogen content is 0.10–0.18%. NMVS soil is classified as low carbonate in terms of the amount of CO_2. The low carbonate content is associated with the hydrothermal features of the regime of these types of grey soil and the nature of the source rocks. The pH of the studied NMVS soil samples ranged from 6.53 to 7.80. A chemical analysis performed in the Agrochemical Laboratory in Bishkek in 2020-2021 showed that these soils contained the following compounds: mobile phosphorus compounds at 26.0 mg/kg, exchangeable potassium compounds at 184 mg/kg, and nitrate nitrogen at 31.6 mg/kg.

(2) Light Chestnut (LC) Soil. LC soil is the standard soil type found on the Talas foothill trails, from 1,000 to 1,500 m. Regarding temperature, the belt of LC soil in Northern Kyrgyzstan is located in moderately warm areas. The temperature is above 10°C at 2,700 m and is 20°C at 1,800–2,200 m, respectively.

Atmospheric precipitation is 300–400 mm per year. In terms of mechanical composition, LC soil is predominantly a medium to heavy loam. The proportion of particles <0.01 mm is usually 33–55%. Silt particles (<0.001 mm) make up approximately 7.4–17.6% of the soil. There are also light loamy cartilaginous varieties of LC soil. The LC soil located on the foothill plains is highly susceptible to erosion processes. The carbonate content of LC soil varies considerably, ranging from carbonate-free to highly carbonate, and tends to increase with depth. The CO_2 content in the arable

Figure 1: Representative pictures of the studied crop areas. Field cultivated with beans with northern mountain-valley serozem (NMVS) soil (a), field cultivated with dwarf pear trees with NMVS soil (b), field cultivated with beans with light chestnut soil (c), and fallow ground with bulk chestnut soil (d).

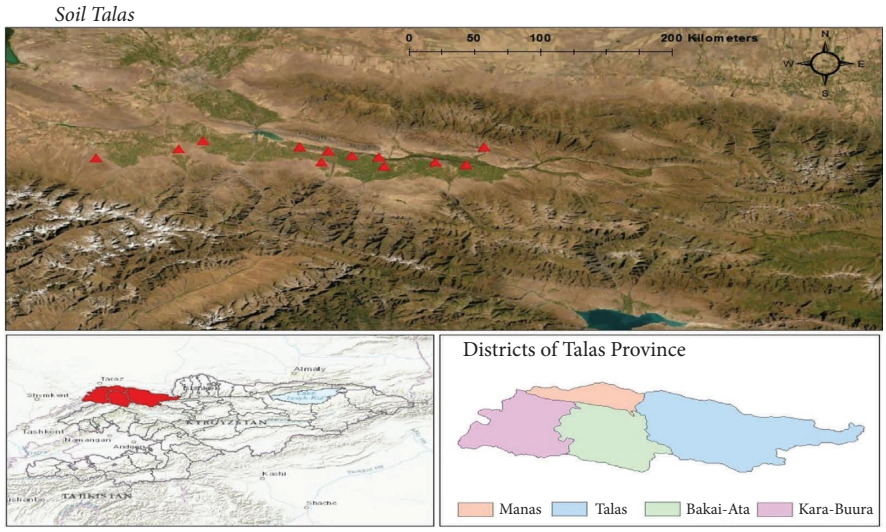

Figure 2: A map of Talas region, red marked sites, where soil samples were selected.

horizon ranges from 0 to 8.2% (average of 2.7%) and in the subsoil from 0 to 11% (average of 4.6%). LC soil is slightly alkaline to alkaline, with a pH of 7.2–8.6. The humus content varies widely depending on the degree of erosion, thickness, and texture. In the arable horizon, it ranges from 0.7 to 4.4% (average of 2.3%), and in the subarable horizon, it ranges from 0.5 to 2.3% (average of 1.5%). The total nitrogen content ranges from 0.10 to 0.30%, and the ratio of carbon to

Table 1: Details of the soil samples collected from the Talas region.

Site	GPS parameters	Soil type	Soil pH	Altitude (m.a.s.l)	Type of land	Air and soil temperature (°C)
TAL-30 Sary-Kuurai (v)	N 42°32′32″ E 72°2′7″	Northern mountain-valley serozem (NMVS)	7.52 ± 0.02	1090	*Phaseolus vulgaris* cultivated	Air: 19 Soil: 10.0
TAL-31 Sary-Kuurai (v)	N42°32′32″ E 72°2′7″	NMVS	7.15 ± 0.01	1090	*Pyrus communis* cultivated	Air: 19 Soil: 7.3
TAL-21 Ozgorush (v)	N 42°30′6″ |E 72°3′16″	Light chestnut (LC)	7.56 ± 0.02	1135	*P. vulgaris* cultivated	Air: 19 Soil: 8.3
TAL-33 Sary-Kuurai (v)	N 42°32′27″ E 72°1′53″	NMVS	8.33 ± 0.12	1089	*P. vulgaris* cultivated	Air: 19 Soil: 10.0
TAL-41 Ak-Dobo (v)	N 42°32′27″ E 72°1′53″	NMVS	6.51 ± 0.01	1085	*P. communis* cultivated	Air: 19 Soil: 15.2
TAL-44 Ak-Dobo (v)	N 42°32′3″ E 71°57′7″	NMVS	6.51 ± 0.01	1085	*P. vulgaris* cultivated	Air: 19 Soil: 13
TAL-54 Leninpol (v)	N 42°32′55″ E 71°56′21″	NMVS	7.13 ± 0.11	1069	*P. vulgaris* cultivated	Air: 19 Soil: 15.1
TAL-58 Bakai-Ata (v)	N 42°32′3″ E 71°57′7″	NMVS	6.51 ± 0.03	1085	*P. vulgaris* cultivated	Air: 19 Soil: 12.8
TAL-70 Bakai-Ata (v)	N 42°31′12″ E 71°49′47″	LC	7.45 ± 0.12	1107	*P. vulgaris* cultivated	Air: 14 Soil: 9.9
TAL-77 Boo-Terek (v)	N 42°35′14″ E 71°45′16″	NMVS	7.8 ± 0.13	922	*P. communis* cultivated	Air: 14 Soil: 7.3
TAL-78 Boo-Terek (v)	N 42°35′14″ E 71°45′16	NMVS	7.46 ± 0.12	922	*P. vulgaris* cultivated	Air: 14 Soil: 9.2
TAL-86 Karasai (v)	N 42°34′44″ E 71°19′29″	LC	7.46 ± 0.12	1085	*P. vulgaris* cultivated	Air: 14 Soil: 7.3
TAL-87 Karasai (v)	N 42°35′23″ E 71°24′27″	LC	7.38 ± 0.13	1075	Bulk soil, nonarable	Air: 14 Soil: 7.3
TAL-88 Cholponbai (v)	N 42°36′54″ E 71°24′39″	NMVS	7.63 ± 0.15	1039	Bulk soil, nonarable	Air: 14 Soil: 7.9
TAL-90 Cholponbai (v)	N 42°36′54″ E 71°24′39″	NMVS	6.88 ± 0.01	1039	*P. vulgaris* cultivated	Air: 14 Soil: 8.8
TAL-97 Cholponbai (v)	N 42°34′14″ E 71°51′23″	NMVS	7.38 ± 0.13	991	Bulk soil, nonarable	Air: 14 Soil: 7.9
TAL-99 Berdike (v)	N 42°28′51″ E 72°15′3″	LC	7.44 ± 0.14	1303	*P. vulgaris* cultivated	Air: 15 Soil: 9.3
TAL-102 Forest of Talas region	N 42°31′19″ E 72°14′11″	LC	7.36 ± 0.13	1242	*P. communis* cultivated	Air: 14 Soil: 8.0
TAL-107 Middle mountain area	N 42°30′32″ E 72°20′36″	LC	7.32 ± 0.12	1313	Natural vegetation, nonarable	Air: 14 Soil: 8.0

nitrogen is 7 : 10. The gross content of P_2O_5 is 0.15–0.23%, and the gross content of K_2O is 2.2–3.1%. The above-mentioned data were obtained from a chemical analysis of soil samples conducted at the Agrochemical Laboratory in Bishkek in 2020-2021.

2.3. Soil Sample Collection. Samples of NMVS and LC soil were collected in autumn from a range of agricultural fields and nonarable areas, as shown in Table 1. Table 1 also shows the soil pH and type, location, altitude, GPS parameters, vegetation type, and agricultural use data for each site. Soil samples were collected at a depth of 0–15 cm from under bean (*Phaseolus vulgaris*) crops and at a depth of 15–30 cm from under pear trees (*Pyrus communis*), also at a depth of 15–30 cm from bulk soil by scraping off the surface material with a sterile spatula and obtaining approximately 100 g of soil per sample. The samples were packed in self-sealing bags and labelled well for future use. Five samples collected from one site were combined, homogenised, and sieved through a 2 mm filter according to cover type. Subsamples were placed on dry ice and stored at −80°C in the laboratory until DNA was extracted. The remainder of each soil sample was subjected to microbiological, chemical, and physical analyses. Samples were stored at 4°C until use.

2.4. Isolation of Microorganisms from the Soil Using Cultivation-Dependent Approaches. From each collected soil sample, 10 g was taken and ground in a sterile porcelain mortar for 5 min in aseptic conditions. After grinding, the soil sample was washed with sterile water. Then, 1 g of each sample was added to 10 mL of Luria–Bertani broth in a 125 mL flask, and the mixture was buffered with sodium acetate (0.25 M, pH 6.8). The broth was incubated in a shaker at 200 rpm for 4 h at 28°C. After incubation, 1 mL aliquots were spread on nutrient agar (NA) plates and incubated at 27°C for 7–10 days to obtain bacterial colonies [42, 43].

The following media were typically used for the growth and qualitative and quantitative characterisation of the soil microflora: Czapek's medium and potato dextrose agar (PDA) for fungal species (incubated at 25°C for 10–12 days), Ashby's medium for oligonitrophilic microorganisms (namely, acetobacter bacteria), starch-ammonia medium (SAA) for actinomycetes (incubated for 15 days), and meat-peptone agar (MPA), starvation agar, and soil agar for bacterial species. Colonies were continually subcultured on new agar plates until pure cultures were obtained, and they were stored at 4°C until further identification was performed.

The phenotypic and biochemical characteristics of the bacterial isolates were determined with reference to Bergey's Manual of Determinative Bacteriology [44] and classified according to Bergey's Manual of Systematics of Archaea and Bacteria [45]. The morphological characteristics of the fungal isolates were identified using the Principles of Fungal Taxonomy and Illustrated Genera of Imperfect Fungi handbooks and the Mycology Online website [46–48]. Images of bacterial cells and fungal mycelia were obtained using a microscope camera (Motic Images Plus, Version 2.0ML, Quick Start Guide, 163 Series Compound Biological Microscope).

2.5. Isolation of Microorganisms from the Soil Using Cultivation-Independent Methods

2.5.1. DNA Extraction, PCR Amplification, and Sequencing of Bacterial Genes. DNA was extracted from the enrichment cultures during the active phase of microbial growth using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) and an alternative protocol developed by the MO BIO Laboratories. To process the soil samples, 5 g of soil was mixed with 10–30 mL of phosphate-buffered saline (PBS) to create a homogenised slurry. The samples were mixed for 1 h at room temperature and then centrifuged for 5 min at 123 × g. The supernatant was removed and centrifuged at 20,000 × g for 15 min. The supernatant was carefully discarded, and the pellet was resuspended in 1 mL PBS. To extract the DNA, 700 μL of the resuspended pellet was processed. The purified bacteria were incubated in a meat-peptone medium (MPM) for two days at 25°C. The cells were harvested when they were in the early exponential growth phase, and the alternative protocol of the MO BIO Laboratories was used to extract the DNA. Successful DNA extraction was determined by agarose gel electrophoresis (1.0% agarose). Amplification was performed with a MultiGene Thermal Cycler (TC9600-G/TC, Labnet International, Edison, New Jersey, USA), using a 25 μL mixture containing 15 μL of PCR Master Mix (Taq DNA polymerase, $MgCl_2$, deoxyribonucleotide triphosphate, and reaction buffer), 2 μL of each primer, 1 μL of template DNA, and 1 μL of H_2O. The amplification program was performed as follows: 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and 72°C for 7 minutes. The PCR products were electrophoresed in a 1.0% agarose gel and visualized using the BioDoc-It Imaging Systems (Ultra-Violet Products Ltd.) after ethidium bromide staining and sterile water was added as the matrix. Almost full-length fragments of 16Sp RNA genes were amplified using the primers 16S-27F and 16S-907R. Sequence analysis was performed by the Macrogen Company (10F World Meridian Center, Seoul, Korea), and sequences were edited with Applied Biosystems 3730XL sequencers. Only sequences with more than 700 nucleotides were used for diversity analyses. The phylogenetic relatedness among different sites was determined using the cluster environment. The 16S ribosomal RNA (rRNA) gene sequences were deposited in the GenBank and DB of the National Center for Biotechnology Information nucleotide sequence databases.

2.5.2. DNA Extraction, PCR Amplification, and Sequencing of Fungi. For DNA extraction, fungal isolates were grown on PDA at 25°C. Approximately 100 mg of mycelium was transferred to a screw-capped 2 mL microcentrifuge tube

containing Lysing Matrix C (MP Biomedicals), and DNA was extracted using the DNeasy Plant Kit (Qiagen). Samples were prepared by homogenization in 400 μL AP1 buffer for up to 1 min at 6 m/s in a FastPrep-2 homogenizer (MP Biomedicals) and briefly centrifuged. After the addition of 4 μL of 100 mg/mL RNase A and 10 min of incubation at 65°C, the standard protocol provided by the manufacturer was followed. Purified DNA was eluted in 100 μL elution buffer AE and stored at −20°C. The following DNA sequences were amplified from fungal DNA samples using standard Taq DNA polymerase (New England Biolabs) using the PCR primers and applying the conditions indicated in the table captioned internal partial sequences of the spacer ITS region in [49] comprising elements ITS1 and ITS4 5′-TCCTCCGCTTATTGATATGC.

The generalized PCR protocol consisted of one initial denaturation step of 95°C for 2 min, 35 cycles of 45 sec at 95°C, 45 sec at the primer-specific annealing temperature, and an elongation step at 68°C for an amplicon-specific elongation time, followed by a 5 min final elongation step at 68°C. PCR product size was controlled by 1, 5% agarose gel electrophoresis. Raw sequence data were combined into a single consensus sequence for each marker using version 6 of the MEGA software package [50].

2.6. Determination of the Number of Microbial Colony-Forming Units (CFU) per Gram of Soil. Under aseptic conditions, 1 g of soil was moistened to a paste state and ground with a rubber pestle for 5 min. To determine the hyphae length of the fungi, 10 g of soil was mixed with 90 mL of water, and an RT-2 tissue microgrinder was used for 5 min at 5,000 rpm. After preliminary dispersion by grinding, the soil suspensions were diluted (from 1 : 2 to 1 : 100,000, depending on the group of microorganisms considered) and plated. Soil bacterial counts were estimated at 10^4 dilutions and fungal population counts at 10^2 dilutions.

The most accurate estimates are obtained when 50–200 colonies of bacteria and actinomycetes and 30–50 colonies of fungi developed on a plate. The seeded plates were incubated at 27-28°C for bacterial and actinomycete growth and at 21–23°C for fungal growth. Counting of the bacterial colonies that grew on NA in Petri dishes was performed after 7 days for the r-strategies group, after 10 days for the K-strategies group, and after 15 days for the actinomycetes. Fungal colonies were counted after 10–12 days of growth on PDA. Colonies were usually counted using a magnifying glass without uncovering the Petri dish. Each counted colony was marked with a dot on the outer surface of the dish bottom using a glass marker for convenience. After determining the number of colonies on all parallel plates, the average number of colonies per plate was calculated.

2.7. Determination of the Presence of Nitrogen-Fixing Bacteria (Azotobacter) in the Soil Samples. The soil lumps method was used to detect *Azotobacter* bacteria in the soil samples. The soil was moistened with sterile tap water until it reached a pasty state. The lumps were laid out in regular rows (according to a stencil) with a microbiological loop or needle, 30 in each Petri dish, on Ashby's medium. Three Petri dishes were used in parallel in triplicate for each soil sample. The dishes were placed in a thermostat-controlled humid chamber. After 4–6 days, the number of soil lumps overgrown with mucous colonies of *Azotobacter* was determined. *Azotobacter* colonies are usually slimy with a doughy consistency, white opaque, and the formation of a brownish pigment is observed with age, appearing on the 4th–6th day of incubation. In terms of their morphology, the cells resemble short, thick rods or cocci, and are often connected in pairs and are surrounded by a capsule. *Azotobacter* colonies should be distinguished from lipomycete yeasts, which form slimy, spreading colonies with a glossy sheen and appear on the 14th–16th day of incubation.

2.8. Statistical Analysis. The Shannon index was used to determine the complete species composition of the bacterial communities, including the abundant rare species, present at the studied sites [51]. The Shannon–Weaver diversity index is based on communication theory. The Shannon function $H^{'}$ measures the uncertainty, corresponds to the entropy concept, and is calculated using the following equation:

$$H^{'} = -\sum \text{pi} \ln \text{pi}, \quad (1)$$

where pi = ni/N (the share of the i-species in the biotope), ni = the number of the i-species (specimen), N = the total number of microbial species, ln = the natural logarithm, Σ is the Greek symbol that means "sum," ln = natural log, and p = the proportion of the entire community made up of species I. The higher the value of $H^{'}$, the higher the species diversity in a particular community. The lower the value of $H^{'}$, the lower the diversity. A value of $H^{'} = 0$ indicates a community with only one species.

The obtained data were statistically processed using SPSS version 25 software (IBM, USA).

3. Results

3.1. Density of Microbial Communities in the Studied Soils. The studied areas mainly consisted of two soil types: NMVS and LC soil, according to the descriptions of Mamytov [40, 41]. The studied sites also included arable land that supports bean and orchard plots, as well as three natural sites. Table 2 shows the density of bacterial, actinomycete, and fungal cells found in soil samples collected from the various study sites.

When the densities of the bacterial populations in the different soil (NMVS vs. LC) and land (plant cover vs. bulk soil) types were compared, it was found that the greatest proportion of CFU was present in soil from the TAL-88 Cholponbai site. At this site, which has NMVS soil and bulk soil without vegetation, the number of CFU/g of soil reached $3.9 \times 10^6 \pm 0.84$. Another two sites, TAL-99 Berdike (LC soil, arable land with beans) and TAL-97 Cholponbai (NMVS soil, bulk soil without vegetation), were also found to contain a significant number of bacterial cells/g of soil, at $1.2 \times 10^6 \pm 0.72$ and $1.03 \times 10^6 \pm 0.93$, respectively. The TAL-77 Boo-Terek village site (NMVS soil, land cultivated

Table 2: Density of bacterial, actinomycete, and fungal cells found at the various study sites.

Site	Soil type	Type of land	Bacterial cells/g of soil	Fungal cells/g of soil	Actinomycete cells/g of soil
TAL-21 Ozgorush village (v)	Light chestnut (LC)	*Phaseolus vulgaris* cultivated	$1.1 \times 10^5 \pm 0.81$	$3.18 \times 10^2 \pm 0.90$	$1.0 \times 10^2 \pm 0.13$
TAL-30 Sary-Kuurai (v)	Northern mountain-valley serozem (NMVS)	*P. vulgaris* cultivated	$5.4 \times 10^5 \pm 0.62$	$6.36 \times 10^2 \pm 0.95$	$15.6 \times 10^3 \pm 0.89$
TAL-31 Sary-Kuurai (v)	NMVS	*Pyrus communis* cultivated	$2.2 \times 10^5 \pm 0.31$	$1.36 \times 10^2 \pm 0.12$	$10.6 \times 10^3 \pm 0.78$
TAL-33 Sary-Kuurai (v)	NMVS	*P. vulgaris* cultivated	$8.8 \times 10^5 \pm 0.40$	$2.72 \times 10^2 \pm 0.32$	$10.4 \times 10^3 \pm 0.25$
TAL-41 Ak-Dobo (v)	NMVS	*P. communis* cultivated	$1.5 \times 10^5 \pm 0.45$	$6.36 \times 10^2 \pm 0.56$	$27.5 \times 10^3 \pm 0.71$
TAL-44 Ak-Dobo (v)	NMVS	*P. vulgaris* cultivated	$3.2 \times 10^5 \pm 0.23$	$6.81 \times 10^2 \pm 0.34$	$21.8 \times 10^3 \pm 0.43$
TAL-54 Leninpol (v)	NMVS	*P. vulgaris* cultivated	$2.8 \times 10^5 \pm 0.17$	$4.54 \times 10^2 \pm 0.73$	$18.1 \times 10^3 \pm 0.42$
TAL-58 Bakai-Ata (v)	NMVS	*P. vulgaris* cultivated	$2.3 \times 10^5 \pm 0.35$	$1.81 \times 10^2 \pm 0.12$	$9.3 \times 10^3 \pm 0.52$
TAL-70 Bakai-Ata (v)	LC	*P. vulgaris* cultivated	$3.7 \times 10^5 \pm 0.67$	$9 \times 10^2 \pm 0.02$	$8.1 \times 10^3 \pm 0.91$
TAL-77 Boo-Terek (v)	NMVS	*P. communis* cultivated	$1.2 \times 10^6 \pm 0.78$	$9 \times 10^2 \pm 0.01$	$16.9 \times 10^3 \pm 0.95$
TAL-78 Boo-Terek (v)	NMVS	*P. vulgaris* cultivated	$1.1 \times 10^5 \pm 0.11$	$4.09 \times 10^2 \pm 0.71$	$35.6 \times 10^3 \pm 0.92$
TAL-86 Karasai (v)	LC	*P. vulgaris* cultivated	$3.1 \times 10^5 \pm 0.12$	$2.72 \times 10^2 \pm 0.23$	$21.8 \times 10^3 \pm 0.91$
TAL-87 Karasai (v)	LC	Bulk soil, nonarable	$1.9 \times 10^5 \pm 0.19$	$3.86 \times 10^2 \pm 0.45$	$11.2 \times 10^3 \pm 0.76$
TAL-88 Cholponbai (v)	NMVS	Bulk soil, nonarable	$3.9 \times 10^6 \pm 0.84$	$4.09 \times 10^2 \pm 0.61$	$21.8 \times 10^3 \pm 0.92$
TAL-90 Cholponbai (v)	NMVS	*P. vulgaris* cultivated	$1.8 \times 10^5 \pm 0.91$	$1.81 \times 10^2 \pm 0.05$	$3.7 \times 10^3 \pm 0.34$
TAL-97 Cholponbai (v)	NMVS	Bulk soil, nonarable	$1.03 \times 10^6 \pm 0.93$	$1.81 \times 10^2 \pm 0.43$	$4.3 \times 10^3 \pm 0.21$
TAL-99 Berdike (v)	LC	*P. vulgaris* cultivated	$1.2 \times 10^6 \pm 0.72$	$2.27 \times 10^2 \pm 0.34$	$14.3 \times 10^3 \pm 0.31$
TAL-102 Orchard in Talas region	LC	*P. communis* cultivated	$4.8 \times 10^5 \pm 0.36$	$7.72 \times 10^2 \pm 0.72$	$15.1 \times 10^3 \pm .56$
TAL-107	LC	Natural vegetation, nonarable	$1.6 \times 10^5 \pm 0.21$	$2.72 \times 10^2 \pm 0.32$	$21.2 \times 10^3 \pm 072$

with dwarf pear trees) was also characterised by a significant bacterial content ($1.2 \times 10^6 \pm 0.78$ bacterial cells/g of soil). The remaining sites were found to contain low levels of bacterial cells, numbering only a few thousand cells per g of soil (Figure 3).

The studied soils were characterised by a lower content of microscopic fungi than bacteria and actinomycetes. The sites most densely populated with fungal cells were TAL-70 (LC soil, cultivated with beans), TAL-102 (LC soil, fruit nursery, and pear seedlings), TAL-77 (NMVS soil, cultivated with dwarf pear trees), TAL-44 (NMVS soil, cultivated with beans), and TAL-30 (NMVS soil, cultivated with beans). The rest of the sites were characterised by lower fungal cell content (Figure 4).

The actinomycete content of the soils was found to be lower than that of all bacteria. The most significant densities of actinomycete spores were found at the following sites: TAL-70 (LC soil, cultivated with beans), TAL-78 (NMVS soil, cultivated with beans), and TAL-41 (NMVS soil, cultivated with pear trees). Moderate actinomycete cell densities were found at TAL-44 (NMVS soil, cultivated with beans), TAL-86 (LC soil, cultivated with beans), and TAL-88 (NMVS soil, bulk soil) (Figure 5).

The quantity of free-living, nitrogen-fixing *Azotobacter* species in the soil samples was determined by overgrowing soil lumps with colonies of these bacteria (Figure 1S). Applying this simple method across several replicates made it possible to compare the presence and enrichment of *Azotobacter* species in the studied sites and soil types, which serves as an indirect bioindicator of soil fertility. Only two sites (TAL-107 and TAL-97) were found to have low levels of nitrogen-fixing bacteria, both below 50%. The remaining sites were enriched with nitrogen-fixing bacteria, by up to 100%.

Azotobacter levels were low at sites with LC soil and without vegetation. The soil at these sites does not contain enough phosphorus and other elements; that is, its fertility is low (Figure 6).

3.2. Microbial Composition of the Studied Soils Determined Using Cultivation-Dependent Approaches. The bacterial diversity of the NMVS and LC soils was represented by two phyla: Firmicutes and Proteobacteria (Figure 7(a)). Firmicutes species made up < 80% of the bacteria in the NMVS soil samples and 70% of the bacteria in the LC soil samples. Such compositions were observed in samples from almost all sites and were found to not depend on the plant cover or land use practice during the autumn period (Figure 2S).

In terms of the bacterial genera present in the soil samples, an abundance of spore-forming bacteria from the *Bacillus* genus (80–85%) was found in NMVS soil samples. Non-spore-forming species from the *Pseudomonas* (5.0%), *Enterobacter* (5.0%), and *Staphylococcus* (5.0%) genera were also detected, as well as autochthonous microflora, such as *Nocardia* species (5.0%) (Figure 7(b)). The proportions of the pseudomonads and autochthonous microflora were higher in the LC soil samples than in the NMVS soil samples.

The species diversity of the soil fungi differed slightly between the two studied soil types. Representatives of nine known genera were found in the NMVS soil samples, including common saprophytic species from the *Aspergillus* and *Penicillium* genera involved in the decomposition of plant residue and pathogenic *Fusarium* species. This was expected because the soil samples were from sites used for the monoculture of beans (Figures 3S–10S).

In the LC soil samples, fungi from five genera were detected. These soil samples were dominated by species from the *Fusarium* and *Penicillium* genera (Figures 8(a) and 8(b)).

3.2.1. Presence of Actinomycetes in the Studied Soils. The actinomycetes are a group of oligotrophic microorganisms that give rise to slow-growing colonies on the surface of a starch-ammonia medium. *Actinomyces* colonies derived from the studied soils were classified into five sections according to their aerial and substrate mycelium morphology and colour [52]. In both soils, representatives of the Albus section dominated, reaching $33.0 \pm 0.71\%$ to $35.0 \pm 0.41\%$.

Cinereus-type species were the second most prevalent, accounting for $20.0 \pm 0.12\%$ to $25.5 \pm 0.31\%$. They were more abundant in the LC soil samples than in the NMVS soil samples. Representatives of the *Roseus* section were also prominent, reaching $19.0 \pm 0.72\%$ and $20.0 \pm 0.23\%$ in the NMVS and LC soil samples, respectively. Species from the *Azureus and Helvolo-Flavus* sections were found in medium and low proportions.

A rich biodiversity of actinomycetes was found at the TAL-44 site in soil-supporting beans (soil pH = 7.27), the TAL-102 site in soil-supporting pear trees, and the TAL-107 site in soil-supporting natural vegetation. The proportions of the actinomycetes belonging to each of the five sections found in the two soil types are shown in Figures 9(a) and 9(b), respectively.

3.3. Microbial Composition of the Studied Soils Determined Using Cultivation-Independent Methods

3.3.1. Bacterial Diversity in NMVS Soil Samples. When the bacterial composition of the two types of soils located under different vegetation was examined via direct analysis of the 16S rRNA gene, rich bacterial biodiversity was found; such biodiversity was not detected when classical cultivation on a nutrient medium was performed. Applying this approach (i.e., examining genes) to the analysis of soil bacteria made it possible to determine the presence and level of bacterial biodiversity in the different soil types and under different vegetation types. For example, the 16S rRNA analysis revealed that *Bacillus* species prevailed in NMVS soil samples from fields with a monoculture of beans, namely, *Bacillus subtilis* (including *B. subtilis* ssp. subtilis), *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus licheniformis*, *Bacillus mojavensis*, and *Bacillus pumilus* (Figure 10). Among the identified species were industrially important species, such as B. atrophaeus and B. amyloliquefaciens. B. amyloliquefaciens is a root-

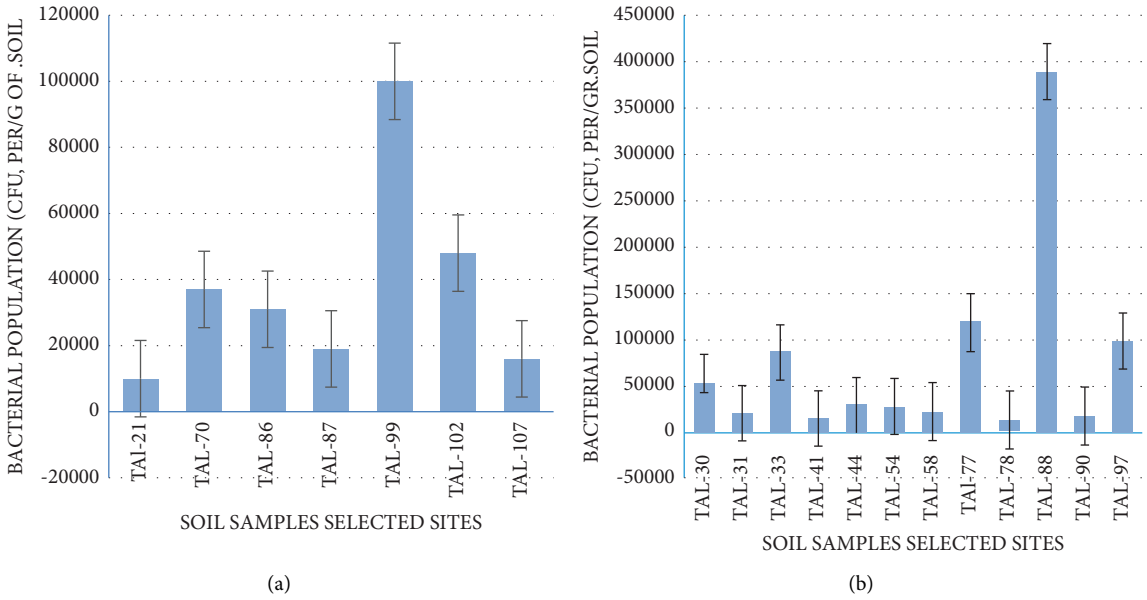

Figure 3: Bacterial colony-forming units (CFU) per g of soil found in light chestnut soil (a) and northern mountain-valley serozem soil (b) samples. Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leq 0.05$.

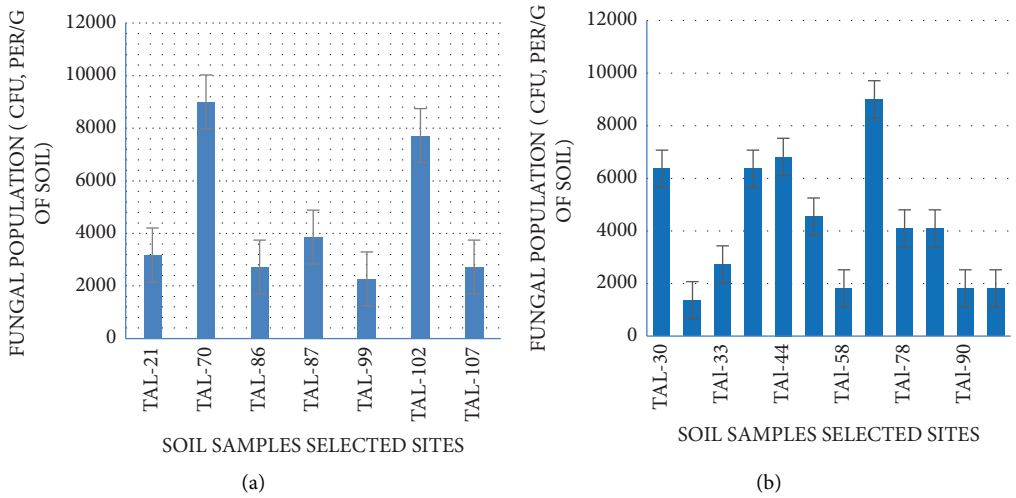

Figure 4: Fungal colony-forming units (CFU)/per g of soil found in light chestnut soil (a) and northern mountain-valley serozem soil (b) samples. Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leq 0.05$.

colonising biocontrol bacterium used to fight some plant root pathogens in agriculture [53]. The second most predominant group consisted of species from the *Brevibacterium* genus (order: Actinomycetales). These bacteria inhabit insects and decaying organic matter and can metabolise carbon and nitrogen [54].

The same *Bacillus* species were found at the sites where pear trees were grown, including *B. atrophaeus, B. pumilus, B. amyloliquefaciens*, and *B. tequilensis* (Figure 11). In other words, the soil bacterial communities in the soils supporting pear trees were similar in composition to those in the soils supporting bean plants. Regardless of the vegetation type, the soil type and pH may affect the levels of the different bacterial species. For example, more dead organic residue enters the ground in autumn.

While spore-forming bacteria were also dominant at sites without vegetation, the species differed from those found in the soil under bean plants and pear trees. Specifically, the *Bacillus* species were different at sites with NMVS soil without vegetation from those at sites with cultivated soil. In addition, classical ammonifiers were found, such as *Bacillus mycoides*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus albus*, and *Bacillus wiedmannii* (Figure 12). These can grow in temperatures from 3°C to 45°C,

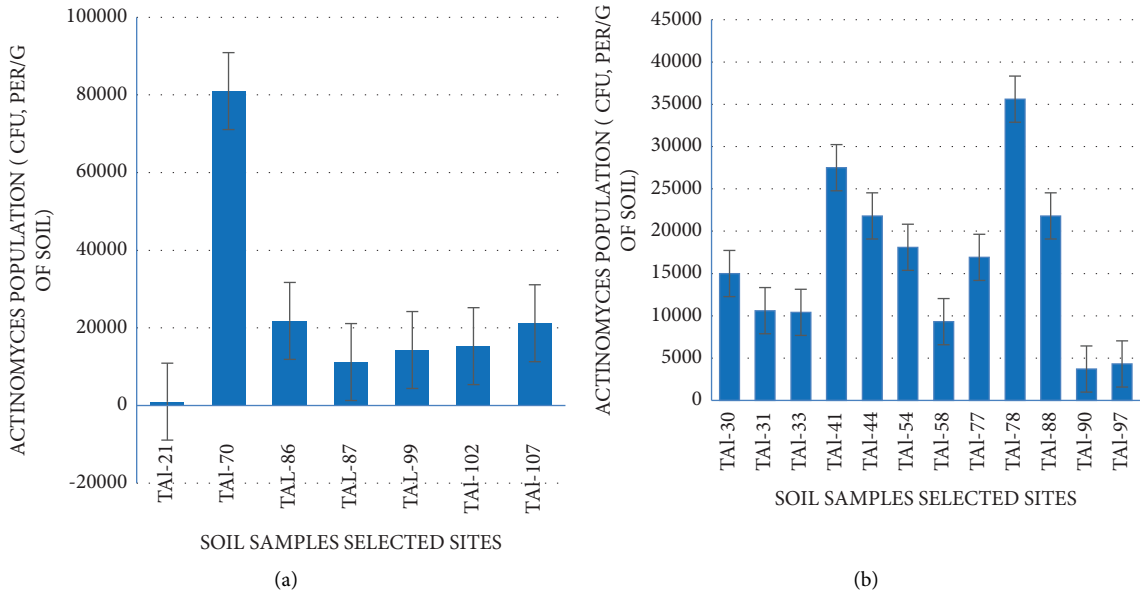

Figure 5: Actinomyces colony-forming units (CFU)/per g of soil found in light chestnut soil (a) and northern mountain-valley serozem soil (b) samples. Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leq 0.05$.

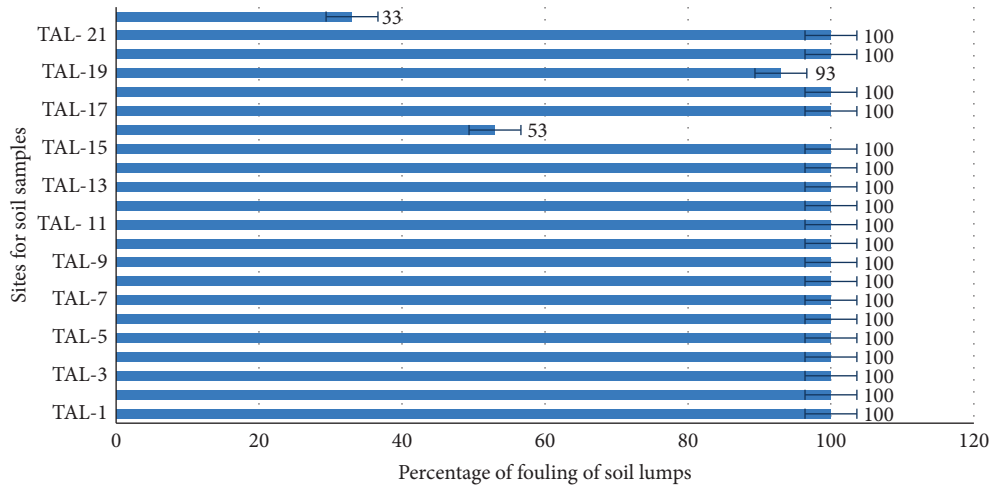

Figure 6: *Azotobacter* bacterial content at the studied sites. Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leq 0.05$.

with the optimum around 30°C, and produce a broad set of enzymes that support the modification of organic matter and substances in soil. *B. megaterium* strains are considered halophiles and grow in a saline (2–10% NaCl) environment. Thus, these bacteria are adapted to different soil compositions [53].

Spore-forming bacteria are credited with playing the leading role in soil formation processes due to their ability to metabolise organic residue that enters the soil. In most of the studied sites, *Bacillus* species were found to dominate.

Non-spore-forming bacterial species from the Proteobacteria phylum and from a range of genera were detected; in some locations, they were found to be abundant (Figure 13). In soils under bean plants, *Ochrobactrum* species were dominant. According to other authors, these bacteria are found widely in the environment, including soil, plants, and animals [55]. Non-spore-forming *Alcaligenes* species were also frequently found. *Alcaligenes faecalis* has been identified as an active ammonifier due to its ability to decompose urea to ammonia and has adapted to alkaline environments, including soil [56].

In addition, pathogenic and opportunistic bacteria were detected in the soil under bean plants, such as *Stenotrophomonas* spp. and *Agrobacterium* species.

3.3.2. Bacterial Diversity in LC Soil Samples. At the sites with LC soil, representatives of the Proteobacteria phylum prevailed over those of the Firmicutes phylum. It was found that most of the Proteobacteria species were different from those found at the sites with NMVS soil. A wide range of aerobic heterotrophic bacterial genera were represented, such as *Achromobacter*, *Alcaligenes*, and *Aminobacter*, as well as the

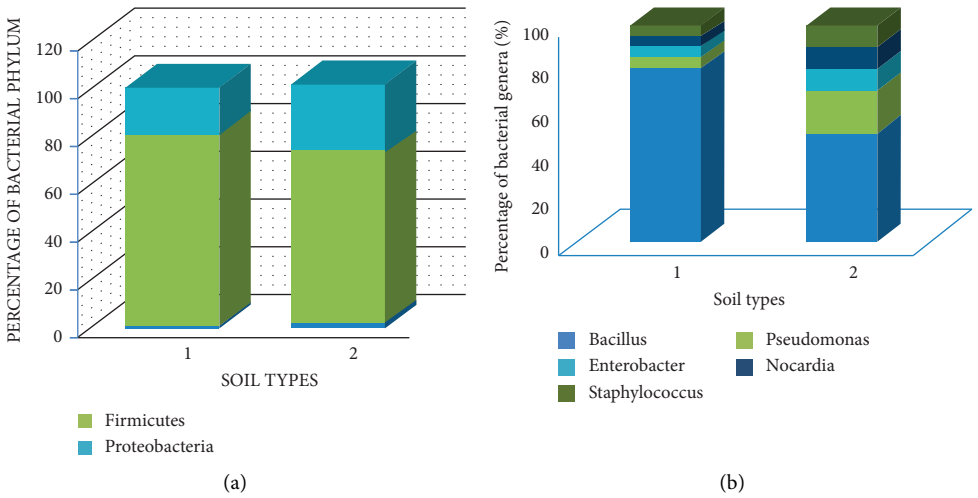

FIGURE 7: The bacterial phyla and genera found in the soil samples. Percentage of bacteria from the Firmicutes and Proteobacteria phyla in the northern mountain-valley serozem (NMVS) (1) and light chestnut (LC) (2) soil samples (a) and percentage of bacteria from five genera in the NMVS (1) and LC (2) soil samples (b). Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leqslant 0.05$.

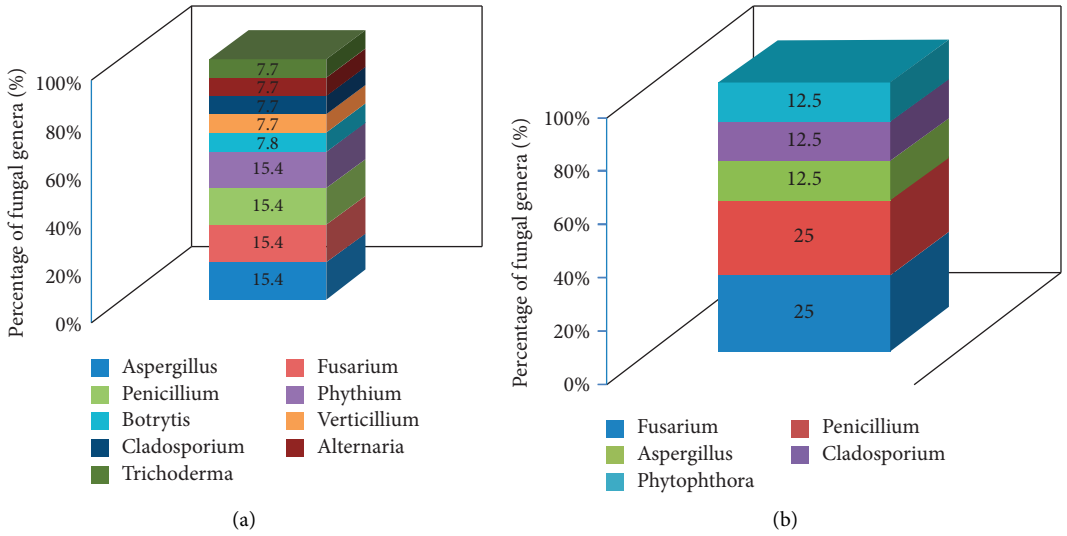

FIGURE 8: Fungal genera proportion found in NMVS soil samples (a) and fungal genera proportion found in LC soil samples (b). Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leqslant 0.05$.

Gammaproteobacteria and Betaproteobacteria classes. Most of the bacterial species were from the *Achromobacter* genus (Figure 14(a)). At the sites with LC soil, most of the Firmicutes species also differed from those found at the sites with NMVS soil (Figure 14(b)).

As the results show, LC soil under bean plants was dominated by bacteria from the Alcaligenaceae family and the Achromobacter genus. These bacteria are heterotrophic, rod-shaped, Gram-negative and are isolated from water, soil, humans, and other animals. They decompose many organic substances, including polychlorinated biphenyls, using them as sole carbon and energy sources. In addition, Betaproteobacteria and Gammaproteobacteria species were found to be widely distributed and abundant in the soil at these sites.

When analysing the biodiversity of the bacterial communities according to the Shannon index, it was found that there was greater biodiversity at the sites with NMVS soil than at those with LC soil. NMVS soil samples collected from under bean plants had a higher sensitivity biodiversity index value (6.21) than those collected from under pear trees (3.9). Even in fallow soil, significant biodiversity was detected (Figure 15).

3.3.3. Biodiversity of Streptomyces Species in NMVS Soil Samples. Actinomycetes are an essential part of soil microflora. Their ability to adapt to various conditions allows them to metabolise a broad group of substances as energy

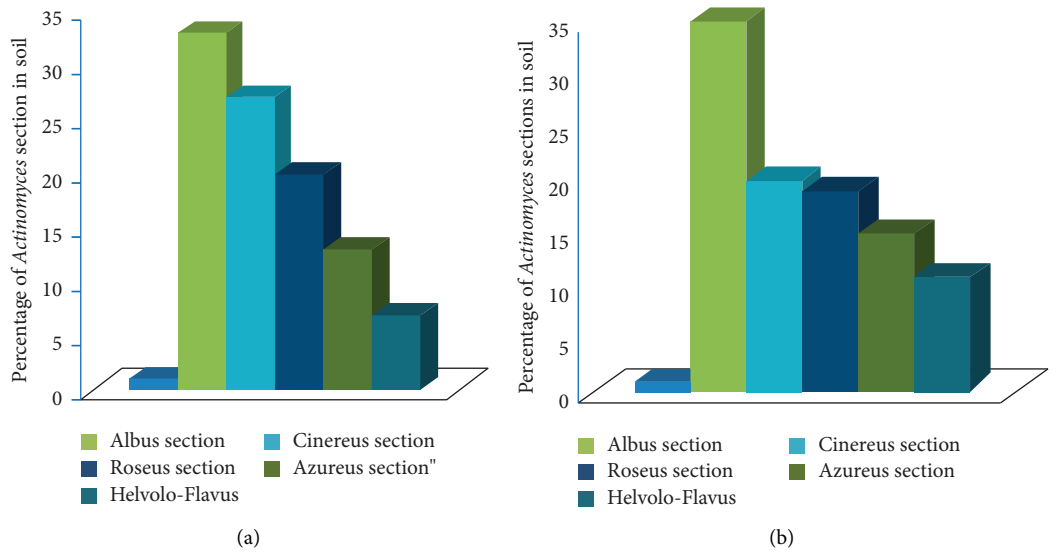

Figure 9: The proportions of the actinomycetes belonging to each of the five sections found in the two soil types. The *Actinomyces* diversity is found in the northern mountain-valley serozem soil samples (a) and in the light chestnut soil samples (b). Values are given as mean ± SD, $n = 3$, and were significantly different at $P \leqslant 0.05$.

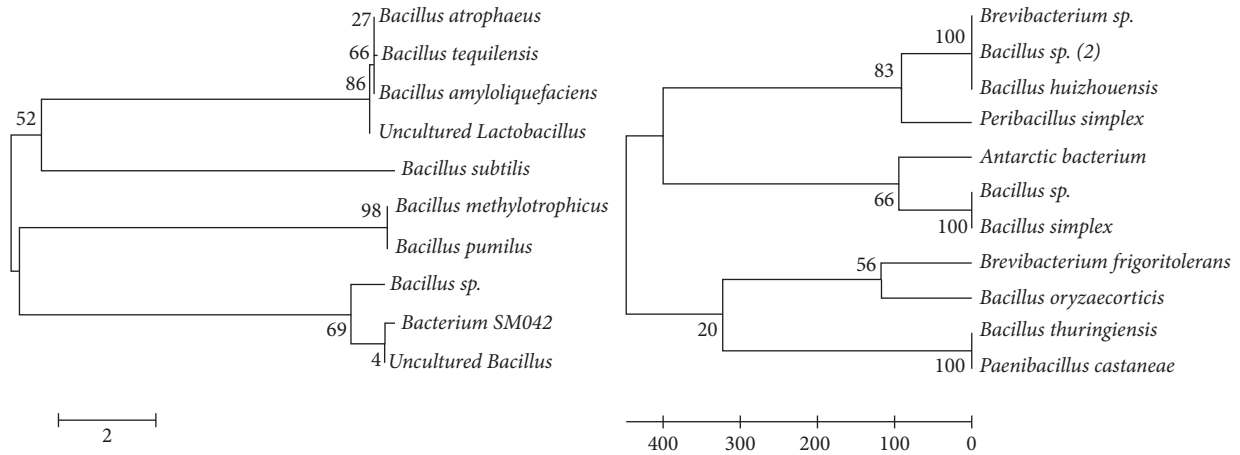

Figure 10: A schematic illustration of the bacterial diversity within the *Bacillus* genus found in the northern mountain-valley serozem soil samples collected from sites cultivated with *Phaseolus vulgaris*. The phylogenetic trees are based on the 16S rRNA sequences and were drawn using the neighbor-joining method. They show the relationships among the isolates and other similar sequences deposited in the NCBI GenBank.

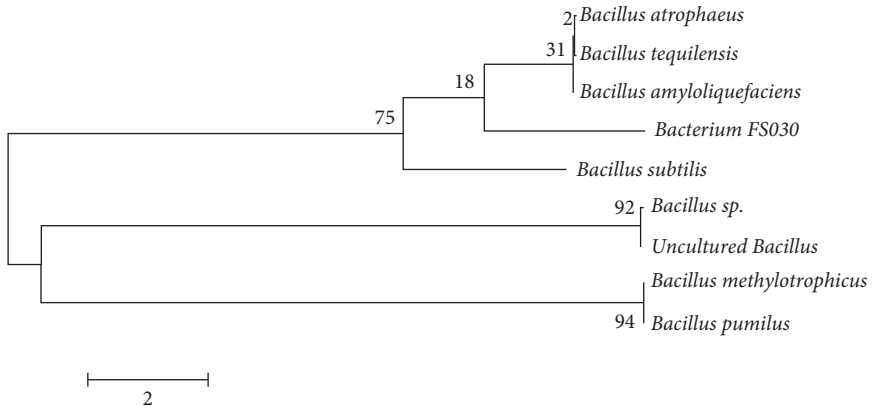

Figure 11: A schematic illustration of the bacterial diversity within the *Bacillus* genus found in the northern mountain-valley serozem soil samples collected from sites cultivated with dwarf pear trees. The phylogenetic trees are based on the 16S rRNA sequences and were drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

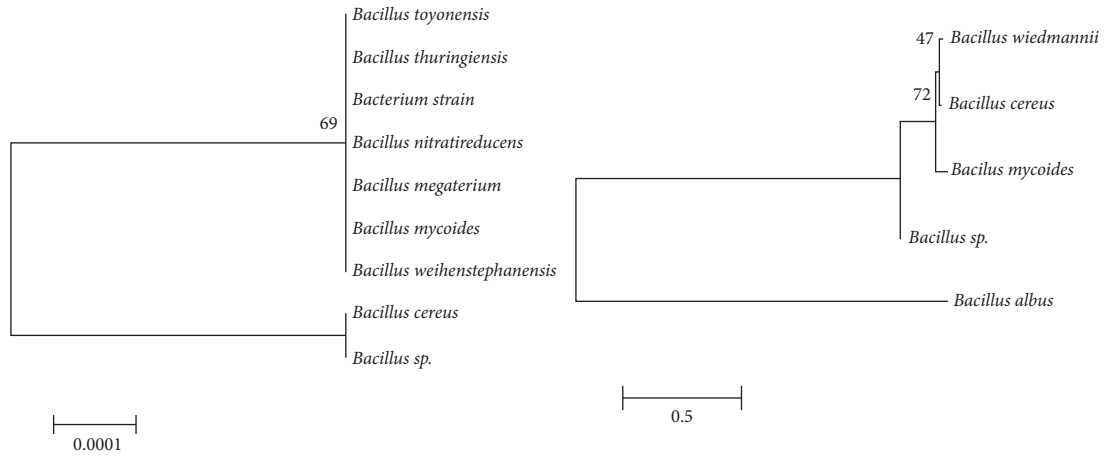

Figure 12: A schematic illustration of the bacterial diversity within the *Bacillus* genus found in the northern mountain-valley serozem soil samples collected from the soil without vegetation. The phylogenetic trees are based on the 16S rRNA sequences and drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

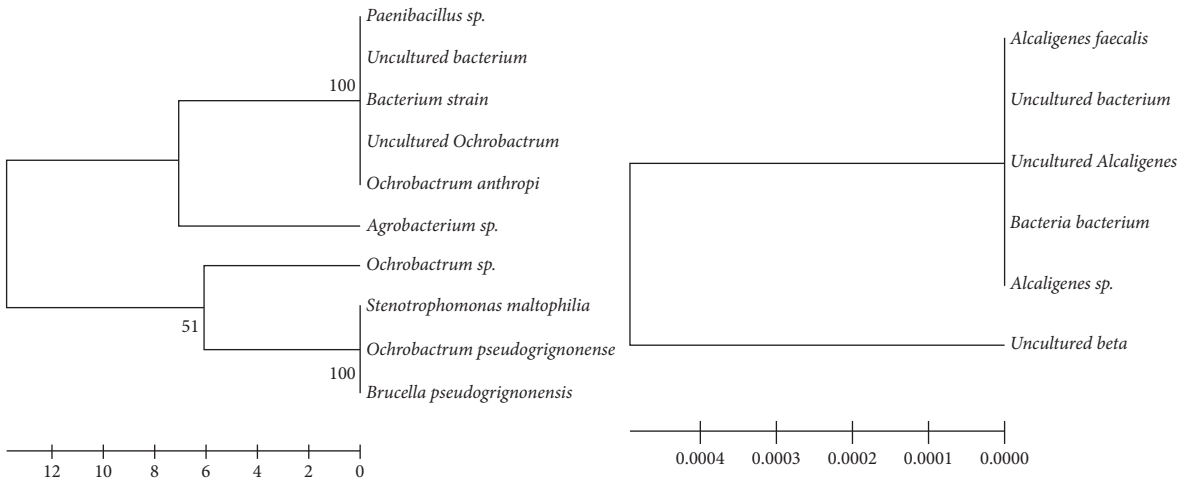

Figure 13: A schematic illustration of non-spore-forming bacteria diversity in the northern mountain-valley serozem soil samples collected from sites cultivated with *Phaseolus vulgaris*. The phylogenetic trees are based on the 16S rRNA sequences and were drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

sources. *Streptomyces* is the largest genus of the Actinobacteria phylum; over 500 species of *Streptomyce* bacteria have been described, and most are found in soil and decaying vegetation [57].

In this study, 16S rRNA gene analysis revealed that there was a rich biodiversity of *Streptomyces* species in the NMVS soil under bean crops, especially at site TAL-44 (Figure 16). *Streptomyces* species are typically found in such soil habitats, and various decomposing organic substances dominate at the sites mentioned above. The presence of decaying vegetation during autumn is a favourable ecological niche for *Streptomyces* species. In addition, antibiotic producers were detected, such as *Streptomyces griseus*, *Streptomyces venezuelae*, *Streptomyces anulatus*, *Streptomyces noisiness*, *Streptomyces omiyaensis,* and *Streptomyces avidinii*.

In the same soil type under pear tree seedlings, the *Streptomyces* biodiversity was less than in the soil under bean plants. Also, even though antibiotic-producing species and heterotrophic species were found, the species composition of the detected *Streptomyces* differed (Figure 17(a)). It was also found that the *Streptomyces* biodiversity was different at sites with LC soil under pear seedlings than at sites with NMVS soil. However, some species found under bean plants in NMVS soil were also found (Figure 17(b)).

In soil without vegetation (LC soil), completely different *Streptomyces* species were found (Figure 18).

The obtained results indicate that the soil and vegetation type influence the quantitative and qualitative composition of the *Streptomyces* population. Among the species found, a large proportion were antibiotic-producing species and species that simultaneously participated in soil-forming processes to decompose organic residue. All these organisms grow in a wide pH range (from 5 to 11) and produce different colour pigments when they grow as colonies.

The Shannon index values were calculated to determine the biodiversity of the *Streptomyces* species in the soil

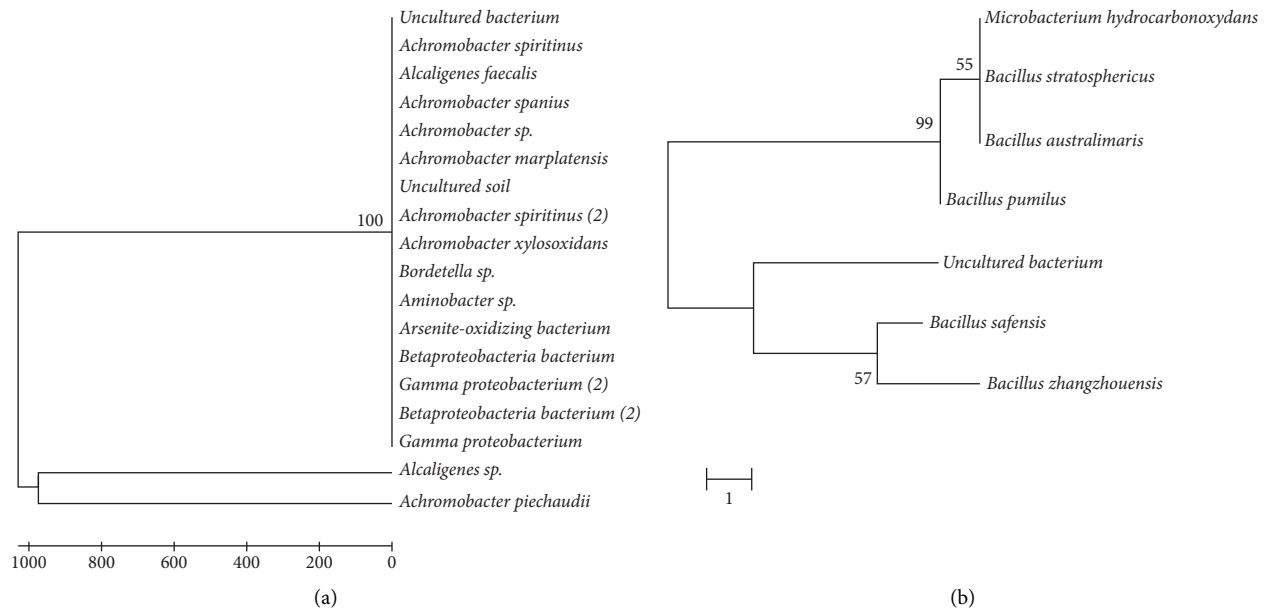

Figure 14: A schematic illustration of bacterial diversity of Alcaligenaceae family (phylum: Proteobacteria) in light chestnut soils with *Phaseolus vulgaris* (a) and bacterial diversity of *Bacillaceae* family (phylum: *Firmicutes)* in light chestnut soils with dwarf pear trees (b). The phylogenetic trees are based on the 16SrRNA sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

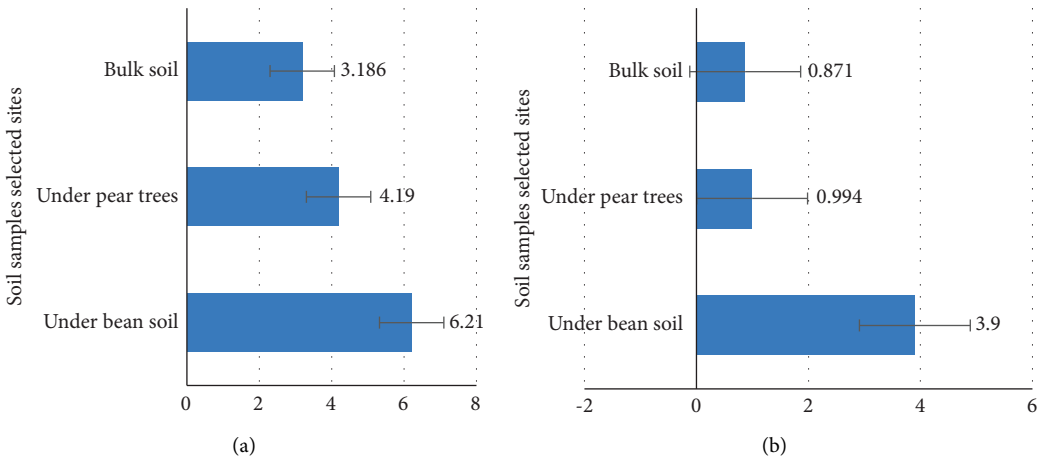

Figure 15: The biodiversity of the soil bacterial communities in the collected samples according to the Shannon index. The left panel (a) shows the values obtained for northern mountain-valley serozem soil samples and the right panel (b) shows the values obtained for light chestnut soil samples.

samples. NMVS soil under bean plants had a higher value (9.2) than LC soil under the same crop (4.21). When the biodiversity of these microorganisms in the soil under pear trees was assessed, it was found that NMVS soil had a higher index (4.44) than LC soil (4.25). In soils without vegetation, the biodiversity index of *Streptomyces* was slightly higher in NMVS soil (1.2) than in LC soil (1.0) (Figure 19).

3.3.4. Biodiversity of Fungi in NMVS Soil Samples. Microscopic fungi contribute to essential processes in soil, particularly the decomposition of plant and animal debris. PCR amplification of internal partial sequences of the spacer ITS region of 18S rRNA allowed us to determine the biodiversity of the soil fungal communities at the studied sites.

At sites with NMVS soil under bean plants, the biodiversity of the soil fungi differed from that at sites with LC soil. In soil under bean plants, the dominant species were from the genus *Fusarium* (division: Ascomycota, family: Nectriaceae), and these were found at almost every site. Despite their predominant role in natural soils as saprophytes, many strains of *Fusarium oxysporium* are plant pathogens, especially in agricultural land. *Geotrichum candidum* (division: Ascomycota, family: Dipodascaceae) was also found in abundance in such soil under bean plants. This species is widespread in soils around the world and is found

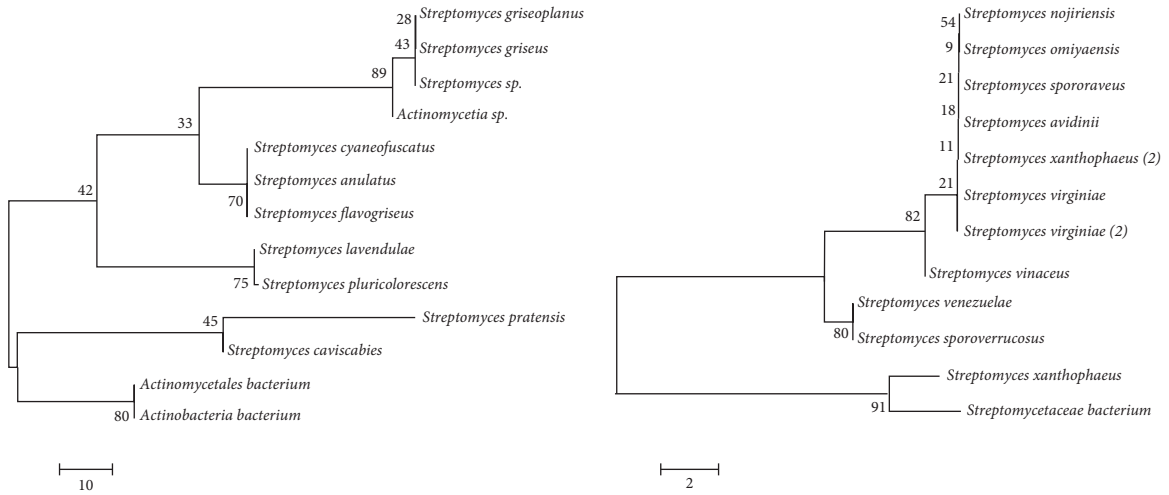

Figure 16: A schematic illustration of *Streptomyces* biodiversity in northern mountain-valley serozem soil samples with *Phaseolus vulgaris*. The phylogenetic trees are based on the 16S rRNA sequences drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

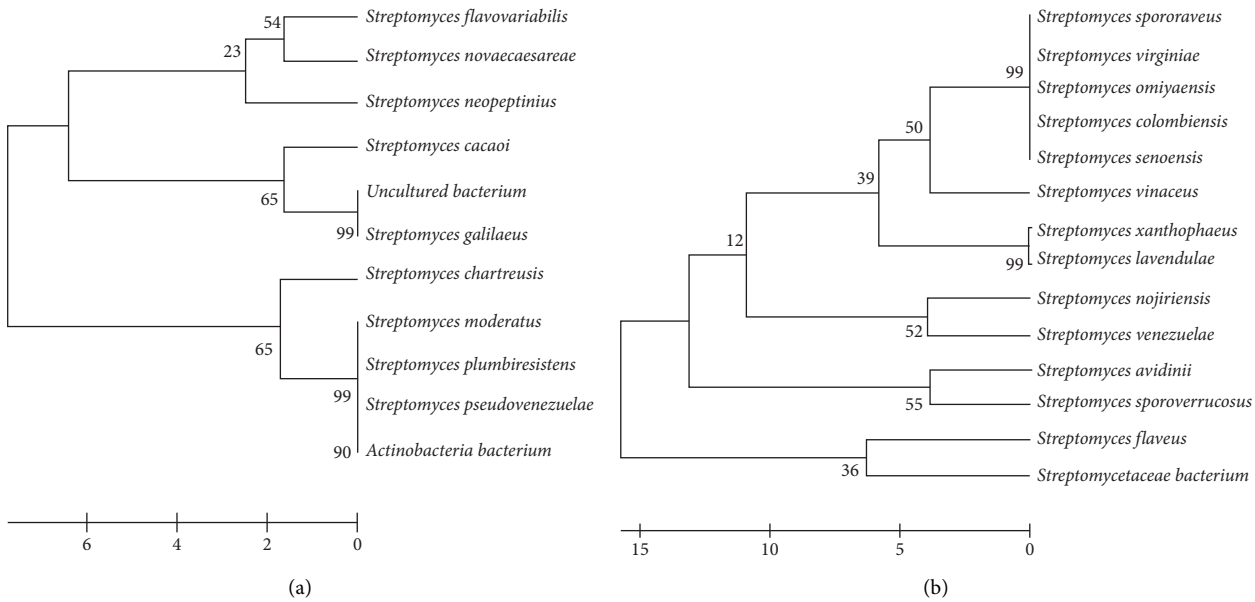

Figure 17: A schematic illustration of *Streptomyces* biodiversity in northern mountain-valley serozem soil with dwarf pear trees (a) and in light chestnut soil with dwarf pear trees (b). The phylogenetic trees are based on the 16S rRNA sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

on all continents. Also, at some sites with bean crops, *Myrothecium verrucaria* and *Albifimbria verrucaria* (division: Ascomycota, family: Stachybotryaceae) were found. These are widespread in soil and plant material and are pathogenic species and saprophytes capable of decomposing cellulose [58] (Figure 20).

The same type of mycoflora, consisting of only *F. oxysporium*, was found in most of the NMVS soil samples (Figure 21(a)). *F. oxysporium* is a ubiquitous soil dweller; it can exist as a saprophyte and decompose lignin and other complex carbohydrates in plant residue [59]. The lack of fungal biodiversity in soils without vegetation may be associated with a lack of plant litter, and fungal species better adapted to such an environment are found in these areas. The soils under pear trees were found to be dominated by fungal species that are specifically associated with fruits, such as *Talaromyces* species (division: Ascomycota, family: Trichocomaceae) (Figure 21(b)).

3.3.5. Biodiversity of Fungi in LC Soil Samples. At almost all the studied sites and in both types of soils, fungi from the *Fusarium* genus dominated. In cultivated areas, pathogenic species from this genus predominated, followed by pathogenic species from the *Alternaria* genus. Compared to uncultivated sites, sites cultivated with beans were found to

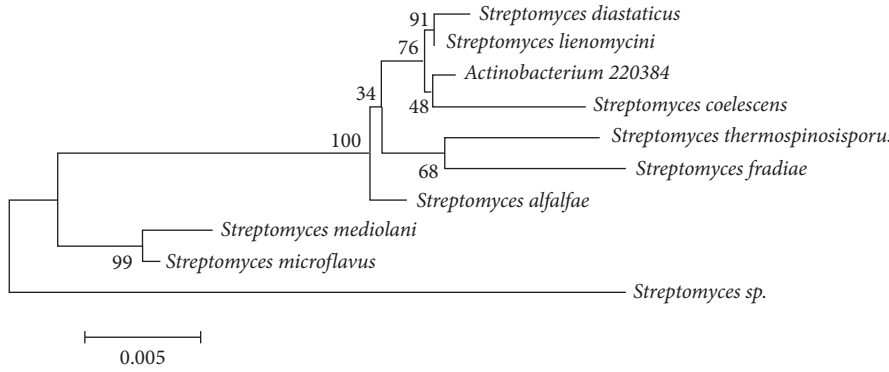

Figure 18: A schematic illustration of *Streptomyces* biodiversity in light chestnut soil without vegetation. The phylogenetic trees are based on the 16S rRNA sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

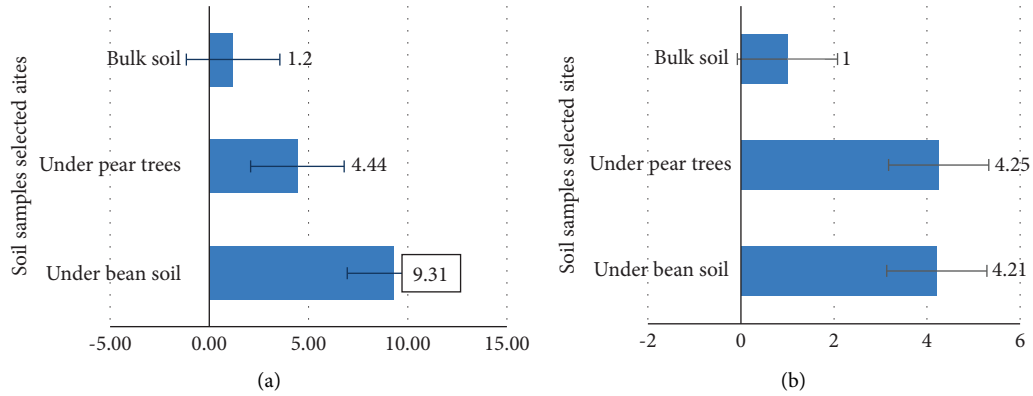

Figure 19: The biodiversity of the soil *Streptomyces* communities in the collected samples according to the Shannon index. The left panel (a) shows the values obtained for northern mountain-valley serozem soil samples, and the right panel (b) shows the values obtained for light chestnut soil samples.

have greater amounts of fungal species from the *Fusarium* and *Alternaria* genera. The results indicate that monoculture farming over a long period may result in the accumulation of phytopathogenic organisms, including fungi, in the soil (Figure 22).

At sites with LC soil under pear tree seedlings, fungal species from the *Cladosporium* genus (division: Ascomycota, family: Davidiellaceae) dominated. Usually, these fungi are widespread indoors and outdoors and found on living and dead plants. Among them are plant pathogens, fungal parasites, and saprophytes [60].

In addition, species from the genus *Talaromyces* (division: Ascomycota, family: Trichocomaceae) accounted for a significant share of the soil fungi found in samples collected from under pear tree seedlings (Figure 23).

In LC soil without vegetation, only *Penicillium* species were found in abundance (Figure 24).

It is well known that *Penicillium* is one of the most widespread genera of fungi in the world, with representatives found in a wide variety of places: in soil, on plants, in the air, indoors, on food, and in the sea. Species of this genus are saprotrophs and weak plant parasites. However, only a few species have been recorded in the rhizosphere soil [61].

When the biodiversity of the soil fungi was analysed, it was found that the Shannon index was higher for sites with LC soil under pear trees (4.188) than for sites with NMVS soil under the same crop (3.9). For soils under bean plants, the Shannon index was higher for sites with LC soil (4.059) than for sites with NMVS soil (3.86). In terms of the bulk soil samples, sites with LC soil had a higher index (2.889) than those with NMVS soil (0.964) (Figure 25).

Thus, based on the obtained results, it can be suggested that, among all the abiotic and biotic factors that affect the formation and function of the individual groups of microorganisms in the plant rhizosphere, the vegetation type has the most significant impact (≤60%). This is followed by soil pH and soil type, which have a ≤30% impact on the biodiversity of microorganisms in the soil. Monoculture farming has a minor influence (approximately 10%) on the biodiversity of the soil microbial species (Figure 26).

4. Discussion

Soil microflora decompose organic substances and generate valuable forms of humus in the deep layers of the earth; hence, they play key roles in maintaining the soil's structure

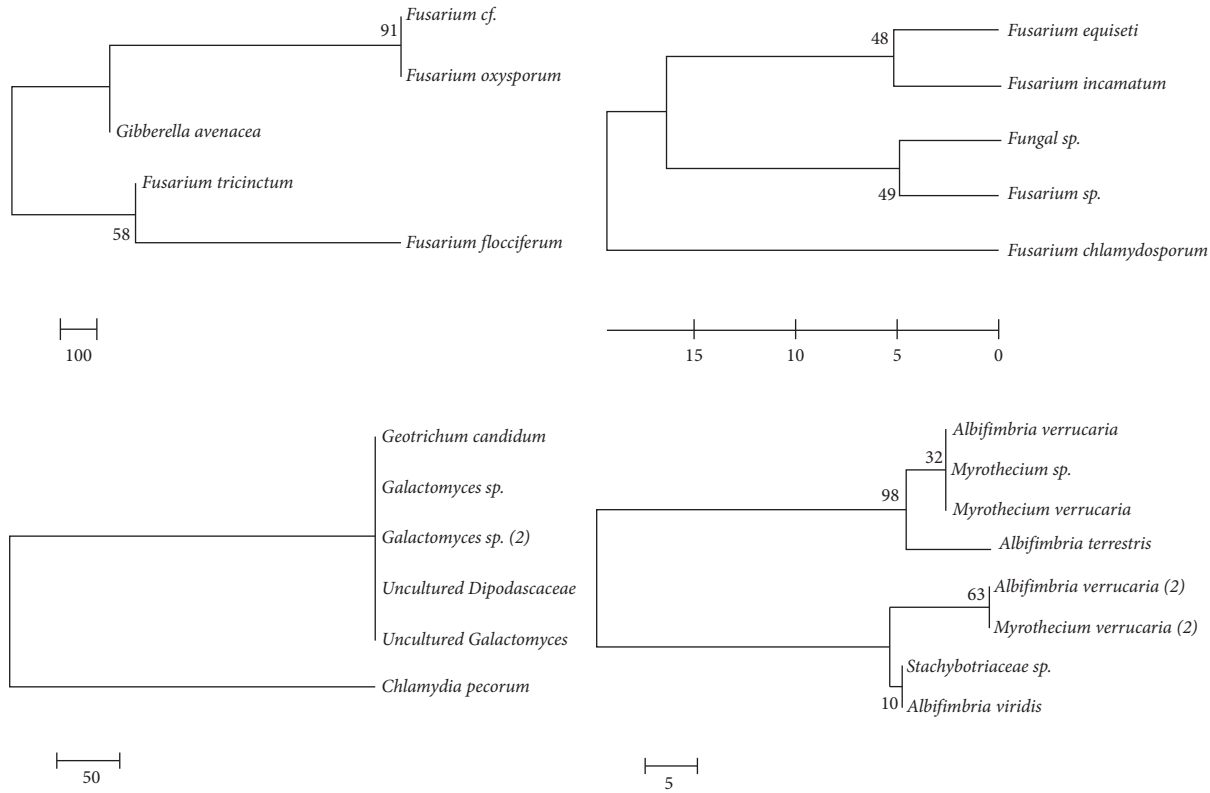

Figure 20: A schematic illustration of microscopic fungi biodiversity in northern mountain-valley serozem soil with *Phaseolus vulgaris*. The phylogenetic trees are based on the ITS region of 18S rRNA sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

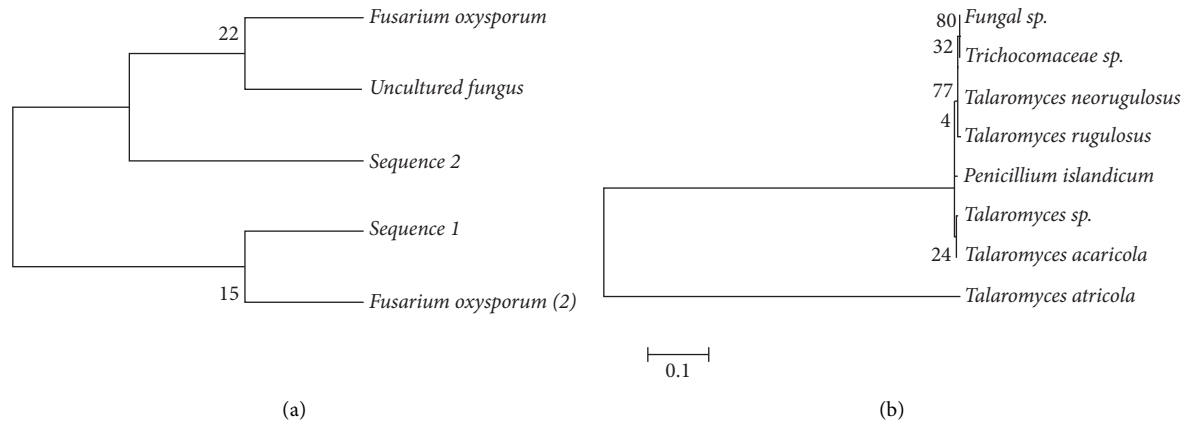

Figure 21: A schematic illustration of microscopic fungi biodiversity in northern mountain-valley serozem bulk soil (a) and with the pear trees (b). The phylogenetic trees are based on the ITS sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

and fertility, as well as the growth and development of plants. Numerous studies have shown that the quantitative criterion of soil fertility is the content of saprophytic and pathogenic bacteria and fungi in specific proportions in one gram of soil [62, 63]. It has also been shown that water availability and soil moisture have a significant effect on the activity of soil microbes [64, 65]. Soil moisture levels are typically highest in spring and autumn due to snow melting and significant amounts of precipitation. Consequently, microorganisms that mineralise plant residue are most active during autumn rather than summer.

In the 1960s and 1970s, several studies were conducted using classical microbiological methods to examine the ecology, distribution, and quantitative and qualitative composition of soil microbes found in various types of soil in Kyrgyzstan [66–69]. The scientists focused their research on the ecological groups of microorganisms present in virgin soils but not in cultivated areas. Almost 50 years have passed

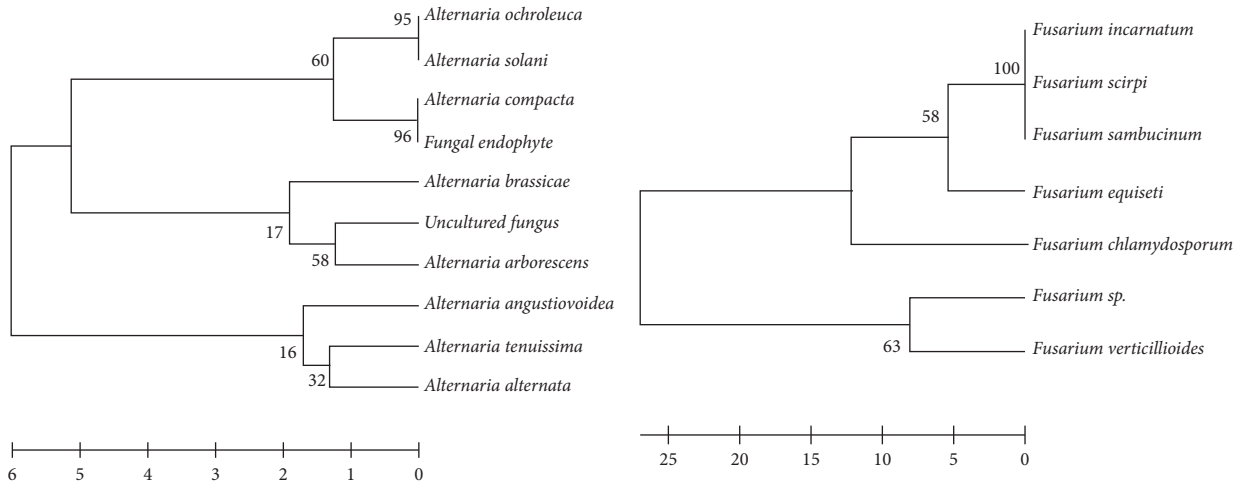

Figure 22: A schematic illustration of microscopic fungi biodiversity in light chestnut soil with *Phaseolus vulgaris*. The phylogenetic trees are based on the ITS sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

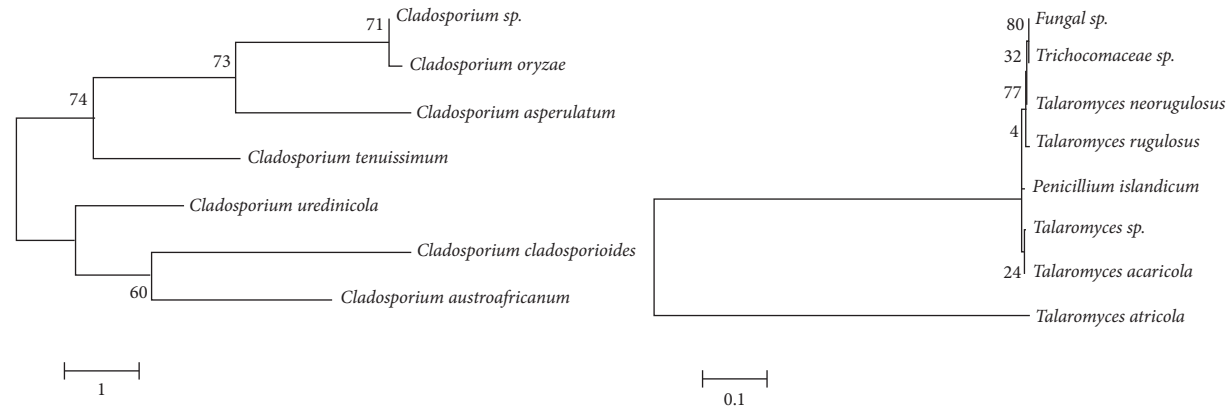

Figure 23: A schematic illustration of microscopic fungi biodiversity in light chestnut soil with pear trees. The phylogenetic trees are based on the ITS sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

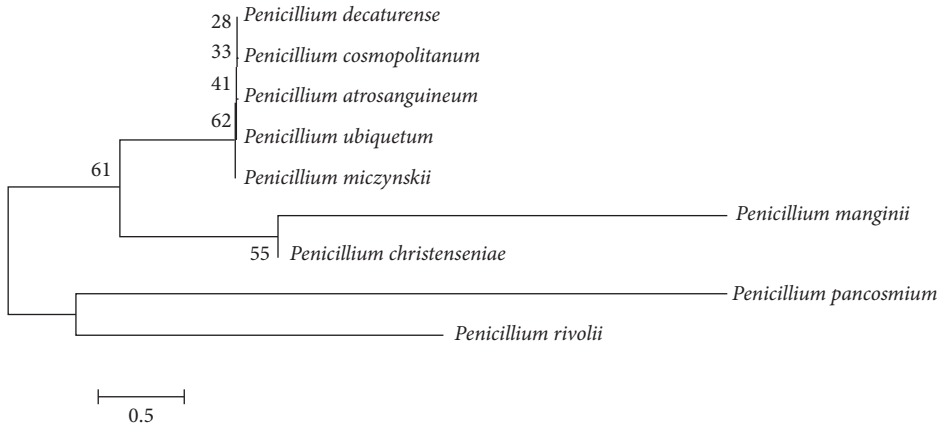

Figure 24: A schematic illustration of microscopic fungi biodiversity in light chestnut bulk soil. The phylogenetic trees are based on the ITS sequences and are drawn using the neighbor-joining method. They show the relationship among the isolates and similar other sequences deposited in the NCBI GenBank.

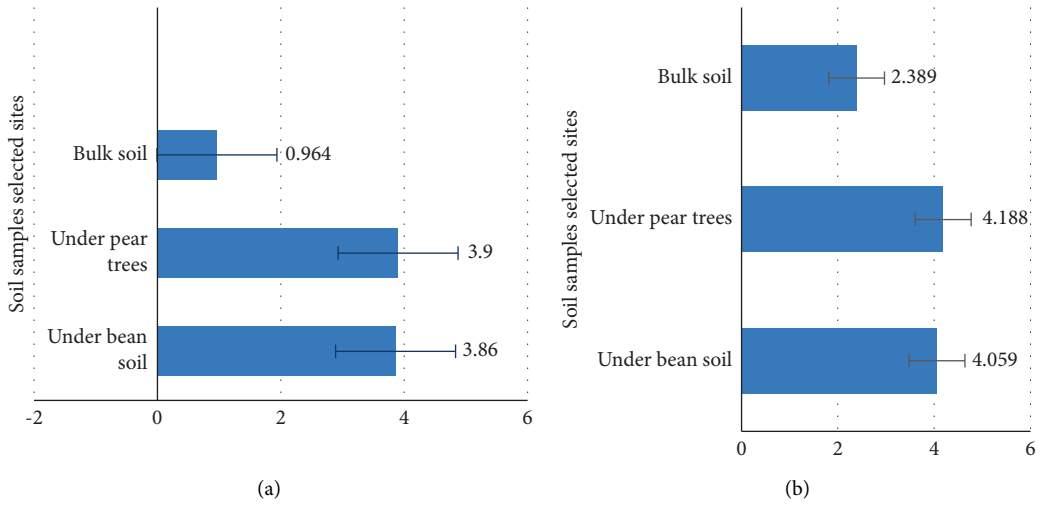

FIGURE 25: The biodiversity of the soil fungal communities in the collected samples according to the Shannon index. The left panel (a) shows the values obtained for northern mountain-valley serozem soil samples, and the right panel (b) shows the values obtained for light chestnut soil samples.

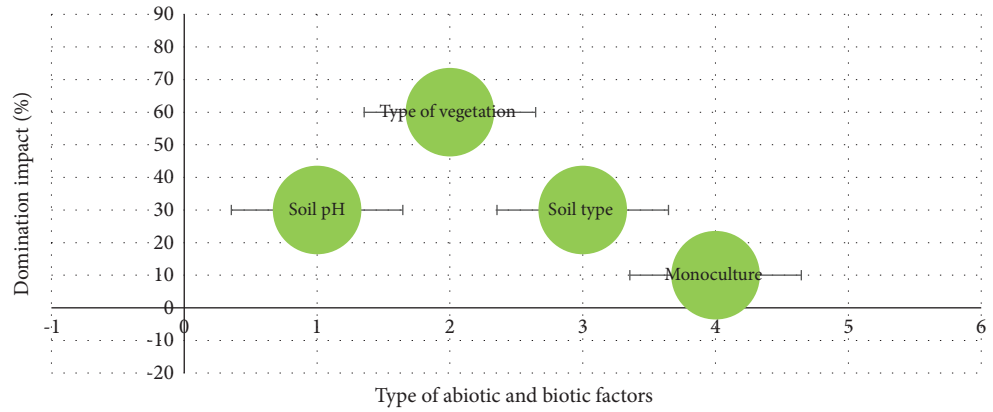

FIGURE 26: The level of impact of four abiotic and biotic factors on the function of microbial communities in arable and bulk soils from the Talas region in the autumn period. Values are given as mean ± SD, $n = 3$, and are significantly different at $P \leqslant 0.05$.

since that time, and changes have occurred in the local and global climate and land use and agricultural practices. In the Talas Valley of Northern Kyrgyzstan, over the past 20 years, beans have mainly been grown in monoculture, with different mineral fertilisers applied.

Given this background, the aim of the present study was to identify and quantify the biodiversity of the microbial communities present in two types of soils (NMVS and LC) in the Talas Valley in the autumn period. In addition, we aimed to compare the soil microbial communities found in soil that supported two different types of crops and in soil without vegetation. Soil samples consisting of a 5–15 cm layer were collected in mid-September (average air temperature = 14–15°C and soil temperature = 8–10°C) from under crops of bean plants and pear tree seedlings. Samples of bulk soil without vegetation were also collected.

Regarding the soil bacterial populations, the results show that the density was two times higher in NMVS soil than in LC soil. The number of bacteria in a gram of LC soil reached 200,000 CFU/g, whereas in NMVS soil, the number reached 400,000 CFU/g. Moreover, bacteria were found at a high density at two sites with NMVS soil without vegetation: TAL-88 (pH 7.63) and (TAL-97) (pH 7.38) (Figure 3). We suggest that the higher bacterial content of the fallow soil compared to that of the cultivated soil is associated with the soil having an optimal pH and the presence of moisture, which support the development of autochthonous microflora, the most permanent members of the soil microbial population. Regardless of the availability of fresh plant residue, these bacteria can intensively reproduce and function.

In contrast to the bacterial communities, the fungal communities in the NMVS and LC soils were present at almost equal density. In the LC soil, high numbers of fungi were recorded under bean plants (pH 7.45) and pear trees (pH 7.36), whereas in the NMVS soil, the fungal density was higher under pear trees (pH 7.8). The average number of fungi was noted in the acidic soils (pH 6.51) under bean plants and pear trees (Figure 4). It is usually accepted that fungal communities prefer acidic environments to neutral

ones; however, in the studied soils, fungal communities were concentrated more in neutral soil than acidic soil, which indicates that the plant cover may specifically impact the fungal density in soil, followed by the pH. It is also worth noting that when plant tissues (e.g. leaves, stems, and roots) are damaged during harvesting or when straw is added, various volatile compounds are released that can have a biocidal effect on rhizosphere microorganisms. In this case, the exudates and organic substances released by the root system of the pear tree were more attractive for the functioning of soil fungi than in leguminous plants.

The actinomycete community density was almost two times higher in LC soil than in NMVS soil under bean plants (pH 7.46) (Figure 5). In LC soil, the microenvironment was the most suitable for this group of microorganisms; actinomycetes are oligotrophs that prefer poor organic compound content and assimilate mineral forms of nitrogen [70, 71]. The rhizosphere effect on oligotrophic microorganisms, such as *Streptomyces* bacteria, was minimal in this study, and this finding aligns with other research that has proved that there is no rhizosphere effect on oligotrophs [72].

In all the studied areas, the two soil types were rich in *Azotobacter* species, indicating that the content of mobile forms of phosphorus, calcium and other elements was sufficient. Only two plots (TAL-107 and TAL-97) with LC soil had low levels of these bacteria, suggesting that LC soil is less fertile than NMVS soil (Figure 6). Others have shown that agroforestry soils contain more *Azotobacter* species than cereal and grassland soils [73, 74].

The standards adopted in soil microbiology indicate that, depending on the presence of humic compounds in the soil and a sufficient amount of organic matter, the number of bacteria either decreases or increases depending on the vegetation type. Several other factors also affect the biomass of saprophytic bacteria, namely, the mechanical composition, genesis, origin, pH, and moisture of the soil, as well as the presence of nitrogen and other elements in the soil. It is also generally accepted that the greater the number of saprophytic bacteria, the more fertile the soil. Hence, soil fertility is primarily determined by the amount of beneficial microflora present. According to Zvyagintsev [42], the average number of microorganisms per gram of fertile soil is as follows: bacteria = 1×10^8–10^9, actinomycetes = 1×10^7–10^8, and fungi = 1×10^5–10^6. Thus, the soils of the Talas region can be classified as moderately fertile.

Performing a 16S rRNA analysis made it possible to determine the bacterial diversity in relation to each soil and vegetation type found at the study sites. In NMVS soil under bean plants and pear trees, sharply different species of spore-forming bacteria were found; however, at some sites, similar species were recorded. At the sites with uncultivated soil, completely different species were found, mainly the classic ammonifying *Bacillus* species: *B. mycoides*, *B. megaterium*, *B. cereus*, *B. albus* and *B. wiedmannii*. This indicates that, depending on the chemical composition of the plant litter, different *Bacillus* species participate as active ammonifiers in the decomposition process. Our data are consistent with those of other studies; these Gram-positive, endospore-forming bacteria inhabit the soil and plant roots and actively participate in plant litter decomposition, particularly in the degradation and transformation of lignin [53, 75].

Regarding the presence of non-spore-forming bacteria, the PCR analysis showed that there were significant differences between the species found in the two soil types and under the different vegetation types. In NMVS soil under bean plants, heterotrophic *Ochrobactrum* species dominated, while other heterotrophic bacteria of the genus *Achromobacter* dominated in LC soil. According to other researchers, these bacteria are found widely in the environment, including soil, the plant rhizosphere, and root exudates; deficient molecular weight carbon substrates are significant drivers of this bacterial diversity [55, 76]. Such results indicate that different soil parameters (pH and humus content) and vegetation exudate compounds could impact the species composition of soil bacteria. In addition, we found pathogenic and opportunistic bacteria in the soil under bean plants, such as *Stenotrophomonas* and *Agrobacterium* species. *Stenotrophomonas* spp. are found mainly in close association with plants, have an essential ecological role in the nitrogen and sulphur cycles, and can engage in beneficial interactions with plants, promoting growth and protecting plants from attack [77, 78]. Moreover, we found widely distributed and abundant Betaproteobacteria and Gammaproteobacteria bacteria at these sites. These bacteria play essential roles in soil pH maintenance and elemental cycling. In particular, Betaproteobacteria and Gammaproteobacteria species are responsible for the crucial organic carbon, nitrogen, and sulphur cycling processes. The relative abundance of Betaproteobacteria and Gammaproteobacteria species is also positively correlated to the dissolved organic carbon (DOC) concentration, which is a critical environmental parameter that shapes the bacterial community composition [79–81].

RNA analysis revealed that the *Streptomyces* species differed in composition between the soil and vegetation types. Diverse *Streptomyces* populations were found at sites with NMVS soil and bean crops, and different species were detected at the sites with pear trees and without vegetation. It is likely that the bean plant residue in the soil attracted more antibiotic-producing bacteria than the pear tree residue. These data provide further evidence that the composition of the plant residue produced during harvesting influences the soil microbial community. In this case, the results suggest that the type of vegetation affects the soil *Streptomyces* biodiversity. Other researchers have shown that these bacteria play major roles in the cycling of organic matter, inhibit the growth of several plant pathogens in the rhizosphere and decompose complex polymer mixtures in dead plants [82].

To determine the composition of the fungal community at each site, PCR amplification of the ITS region of 18S rRNA was performed. It was found that pathogenic species, such as *F. oxysporium* and *G. candidum*, dominated under bean plants in NMVS soil. Other studies have shown these fungi to be widespread in decaying plant tissues and are the causative agents of sour soft rot in citrus fruits, tomatoes, carrots, and other vegetables [83]. *M. verrucaria* and *A. verrucaria* were also detected in significant amounts in soils

under bean plants. Other researchers have reported that these fungi are pathogenic and decompose cellulose [58]. At sites with LC soil and bean crops, the pathogenic and saprophytic *Alternaria* and *Fusarium* genera dominated, while *Cladosporium* and *Talaromus* species prevailed under pear tree seedlings. Most of these species are soil dwellers and produce thermally stable ascospores; therefore, they are sometimes isolated from pasteurised fruit juices and fruit-based products [83, 84].

The studied bulk soil samples had relatively low fungal biodiversity, with *Fusarium* and *Penicillium* species being the main components. These findings indicate that the type of vegetation and its secreted exudates influence the species of fungi that are found in the rhizosphere microflora, which are responsible for the mineralisation of plant residue. Furthermore, compared to the uncultivated soils, the soils cultivated with beans contained *Fusarium* and *Alternaria* species. Thus, monoculture farming over an extended period leads to the accumulation of phytopathogenic organisms, including fungi, in the soil.

The Shannon index is used as an indicator of biodiversity, and comparisons of the Shannon index values calculated for the different sites showed that there were clear differences in the biodiversity of the rhizosphere microorganisms according to soil and vegetation type. For example, the bacteria analysed in this study were found to prefer NMVS soil over LC soil and soil under an annual plant (i.e., the bean plant) rather than that under a perennial fruit tree (i.e., the pear tree). The Shannon index for *Streptomyces* species was higher for NMVS soil and the pear tree rhizosphere than for LC soil. In soil without vegetation, there was more *Streptomyces* biodiversity in NMVS soil than in LC soil. In terms of fungal biodiversity, the Shannon index for fungal species under pear trees was higher for LC soil than for NMVS soil. In LC soil, these micromycetes preferred bean plants as the vegetation cover over pear trees.

It can also be concluded that the soil microflora includes microorganisms with metabolic and substrate cycling activities that are intense yet relatively slow. The most common members of the soil microbial population are autochthonous bacteria, which are physiologically inert species and their activity is helpful for plants as they assimilate soluble forms of nitrogen, preventing them from being washed away.

As noted in other studies, a diverse microbial community can simultaneously support multiple ecosystem functions. Species that do not participate in one specific function at a particular time may contribute at another time or simultaneously to another process. Thus, asynchronous temporal fluctuations in the number of species and their contributions to various ecosystem functions in more diverse communities contribute to better overall ecosystem functioning over time [85–87].

5. Conclusion

Biodiversity and communities of microorganisms differ in soils with annual and perennial plants, which indicates that plant residues have a different chemical composition and different types of microorganisms are involved in their decomposition. The composition of the plant residue produced during harvesting influences the soil microbial community.

A diverse microbial community in the two soil types and under the different vegetation types has significant differences between the species. The most common members of the soil microbial population are autochthonous bacteria, which are physiologically inert species whose activity is helpful for plants as they assimilate soluble forms of nitrogen, preventing them from being washed away.

Among all the abiotic and biotic factors that affect the formation and function of the individual groups of microorganisms in the plant rhizosphere, the vegetation type has the most significant impact (≤60%). This is followed by soil pH and soil type, which have a ≤30% impact on the biodiversity of microorganisms in the soil. Monoculture farming has a minor influence (approximately 10%) on the biodiversity of the soil microbial species.

According to the average number of microorganisms per gram of soil, the results proved that the soils of the Talas region can be classified as moderately fertile.

In the future, such studies will expand and deepen in other northern and southern regions of the country to provide an ecological assessment of the soil fertility and its suitability for cultivating annual and perennial crops.

Data Availability

The data used to support the findings of this study are included within the article and supplementary materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

All authors contributed to the conception and experimental design of the study. Prof. Doolotkeldieva conceived the research, wrote, and edited the manuscript. Dr. Bobusheva and Dr. Konurbaeva performed material preparation, data collection, and analyses. All authors commented on previous versions and read and approved the final manuscript.

Supplementary Materials

Biodiversity of the dominant species of soil bacteria found by classical microbiological research method: Figure 1S: *Azotobacter* colonies on Ashby's medium. Figure 2S: the colonies of spore-forming soil bacteria isolated from Talas region biotypes. Biodiversity of the dominant species of soil fungi found by classical microbiological research method: Figure 3S: phylum: Ascomycota, order: Eurotiales, family: Trichocomaceae, and genus: *Penicillium* spp. Figure 4S: phylum: Deuteromycota, order: Hyphomycetales, family: Dematiaceae, and genus: *Fusarium* spp. Figure 5S: phylum: Oomycota, order: Peronosporales, family: Pythiaceae, and genus: *Pythium* spp. Figure 6S: phylum: Deutromycota, order: Hyphomycetales, family: Dematiaceae, and genus:

Alternaria spp. Figure 7S: phylum: Oomycota, order: Peronosporales, family: Peronosporaceae, and genus: *Phytophthora* spp. Figure 8S: phylum: Deuteromycota, order: Hyphomycetales, family: Moniliaceae, and genus: *Verticillium* spp. Figure 9S: phylum: Oomycota, order: Peronosporales, family: Peronosporaceae, and genus: *Phytophthora* spp. Figure 10S: phylum: Deuteromycota, order: Hyphomycetales, family: Moniliaceae, and genus: *Verticillium* spp. (*Supplementary Materials*)

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