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# Reduced Soil Quality but Increased Microbial Diversity in Cultivated Land Compared to Other Land-Use Types in the Longzhong Loess Plateau

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**Abstract:** Soil microorganisms, as a vital part of terrestrial ecosystems, play a key role in sustaining essential soil functions. However, the impact of cultivated land (CL) on soil quality and microbial communities compared to other land-use types is still unclear. This study investigated the soil quality index (SQI) along with bacterial and fungal communities across various land-use types, including abandoned land, cultivated land, forest land, and grassland, in the Longzhong region of the Loess Plateau. The results showed that CL had the lowest SQI, but the diversity of soil bacterial and fungal communities in CL was significantly higher than that of other land-use types. The relative abundance of Ascomycota in CL fungal communities is significantly higher than that of other land-use types. Soil water content, organic matter, alkaline nitrogen, total nitrogen, and nitrate nitrogen all have an impact on soil bacterial and fungal communities in CL. The diversity of soil bacterial and fungal communities is mainly influenced by pH, nitrate nitrogen, and available phosphorus. This study emphasizes the impact of human activities such as tillage on soil quality, as well as the structure and diversity of soil microbial communities, in cultivated land compared to other different land-use methods.

**Keywords:** land-use types; Longzhong Loess Plateau; soil properties; soil quality index; effecting soil factors; bacterial community; fungal community



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## 1. Introduction

Soil is one of the most diverse and complex environments [1], playing a vital role in material cycling and energy flow within terrestrial ecosystems [2]. Terrestrial ecosystems serve as a significant sink for atmospheric carbon dioxide [3] and are crucial in addressing the issues of global warming and climate change [4]. However, terrestrial ecosystems are susceptible to human activities, particularly land-use changes [5]. Such changes modify both the structure and function of these ecosystems by transforming land cover types. Furthermore, land-use alterations significantly impact the storage of carbon dioxide in soils and disrupt the overall carbon cycle within terrestrial ecosystems [6].

The Loess Plateau in northwestern China ranks among the most severely eroded areas globally [7]. Moreover, it is recognized as one of the most vulnerable ecosystems globally, due to its complex geographical conditions [8], attracting substantial attention from ecological research. Since the 1980s, both the population and cultivated land area in the Loess Plateau region have steadily expanded. During this period, significant shifts in land use have occurred in the region, marked by the widespread conversion of forests and grasslands into agricultural land [6]. However, improper land-use changes can negatively influence the local ecosystem. To mitigate these effects, various measures have been implemented to protect the fragile ecosystem, focusing on preventing soil erosion and land

degradation [9]. The policy of converting farmlands back to forests and grasslands has significantly contributed to land restoration and the protection of the ecological environment, promoting the recovery of degraded areas [10]. Furthermore, the migration of large populations to urban areas has resulted in the abandonment of farmlands, leading to a significant expansion of abandoned land in the Loess Plateau [11]. Undisturbed secondary succession on abandoned land serves as an effective approach for enhancing local soil conditions and restoring ecosystems in the Loess Plateau region [12]. Currently, research in this region is primarily focused on vegetation restoration and water utilization [13,14]. Therefore, studying the effects of different land-use types, especially cultivated land with frequent human interference, on soil properties and microbial communities under the same geographical background can clarify the impact of human farming activities on the terrestrial ecosystem of the Longzhong Loess Plateau, providing detailed data support for the protection of local ecosystems and scientific management and utilization of land.

The interaction between soil properties and microbial communities has been reported to be dynamic, with microorganisms serving as the primary drivers of soil ecological restoration [15]. Different land-use types can influence soil environmental factors, nutrient levels, and biological interactions, all of which subsequently affect the soil microbial communities [16]. Soil physicochemical indicators affected by land use, such as soil conductivity, total nitrogen, organic matter, and heavy metals, have been identified as key determinants of soil microbial communities [17] in the Yellow River Delta, resulting in outstanding variations in soil bacterial communities [18]. Soil pH, soil water content (SWC), and soil enzyme activities were significantly correlated with bacterial abundance, diversity, and community distribution [19]. Similarly, a previous study has indicated that available phosphorus, pH, and heavy metal content significantly affect the structure of soil fungal communities [20]. In a recent study, the soil quality index (SQI) was used as a comprehensive index to assess the quality and health of soil [21]. Considering the complex physical and chemical properties of soil, along with the diverse land-use types in the Loess Plateau region, the impact of different land-use types, especially cultivated land, on soil quality is not yet clear. Therefore, comparing and analyzing the effects of different land-use types on soil physical and chemical properties as well as soil quality can provide a clear understanding of the impact mechanism of human farming activities on soil properties, and provide theoretical guidance for the scientific management and rational utilization of arable land.

Soil microbes are essential parts of terrestrial ecosystems, playing a critical role in the decomposition of plant residues and organic matter, which enhances nutrient cycling, regulates climate change, and supports food security [22]. Bacteria, the most abundant and vital soil microbes, play a crucial role in mineral release, organic matter decomposition, and energy transformation within the soil ecosystem [23]. Conversely, soil fungi establish mutually beneficial symbiotic relationships with plants, enhancing their nutrient uptake capabilities [24]. Furthermore, soil fungi are essential for the decomposition and cycling of nutrients within soil ecosystems [25]. Current research on soil microorganisms primarily examines the impact of fertilization on microbial communities [26], plant–microbial interactions and regulation of ecosystem diversity [27], and the effects of soil factors on soil microbial communities [28]. Only a limited number of studies have explored the effects of various land-use practices on soil physicochemical properties and the characteristics of soil microbial communities, along with their interrelations. Therefore, comparing the relationship between soil properties and soil microbial communities in cultivated land with different land-use types is essential for understanding the interaction mechanisms and preserving balance within terrestrial ecosystems.

This study posits the following hypotheses: (1) Land-use changes in the Loess Plateau of central Gansu will alter the physical and chemical properties of soil, leading to significant changes in SQI. Due to frequent human disturbances such as tillage, the SQI of cultivated land is lower than that of other land-use types. (2) The soil bacterial and fungal communities in cultivated land are different from the other three land-use methods. (3) Cultivated land

has greater microbial diversity compared to other types. To evaluate these hypotheses, four distinct land-use types in the Longzhong Loess Plateau region were selected for analysis. A combination of chemical analyses and high-throughput sequencing techniques was used to assess soil properties and the characteristics of microbial communities across these land-use types. Furthermore, the study elucidated the mechanisms through which soil factors influence microbial communities. This investigation contributes to a deeper understanding of the alterations in soil physicochemical properties and the dynamics of microbial communities resulting from changes in land use within the Longzhong Loess Plateau region. The results of this study will provide a theoretical foundation for the sustainable application and scientific management of cultivated land resources in this region.

## 2. Materials and Methods

### *2.1. Research Area*

The study area is located in Wenfeng Town of Longxi County in Gansu Province, China (104°40′05″–104°40′30″ E, 35°3′10″–35°3′20″ N, altitude: 2180 m a.s.l.). The area has a mean annual temperature ranging from 6 to 7 °C and receives approximately 450 mm of precipitation annually. Rainfall is the sole water source for plant growth in this region, where the soil is classified as secondary loess soils according to Xie et al. [29], characterized as yellowish, loose, and macroporous with a clayey or loamy texture.

### *2.2. Experimental Design*

This research focused on four representative local land-use types: abandoned land (AL), cultivated land (CL), forest land (FL), and grassland (GL) (Figure 1). The AL site was previously employed for cultivation during the 1980s but was abandoned in 2008 following the cessation of agricultural activities. In the past, traditional manual farming methods were used to cultivate wheat on AL, in order to provide a source of food for local residents. For many years, CL has been conducting continuous management under the maize–potato rotation system, planting crops through a combination of animal power and mechanical tillage to achieve economic benefits. Potatoes are planted in the current season, CL has no irrigation conditions, and appropriate amounts of nitrogen and phosphorus fertilizers are applied by local farmers based on their planting experience. FL grows naturally without human disturbance, with *Salix myrtillacea* Andersson and *Nothosmyrnium japonicum* Miq as the dominant species. GL also grows naturally and is grazed year-round, with dominant plants including *Fragaria vesca* L., *Poa pratensis* L., *Anaphalis flavescens* Hand.-Mazz., etc.

### *2.3. Soil Sample Collection and Determination*

In August 2023, soil samples of four different land-use types were collected from a depth of 20 cm using a soil drill with a diameter of 3.5 cm. Three repeated areas were established with different land-use patterns, twenty soil cores were obtained from each area of each land-use type, and ten soil cores were mixed in situ into one composite sample to decrease errors induced by heterogeneity. Thus, 6 composite soil samples were obtained in each land-use type (3 areas × 2 composite samples). In this study, a total of 24 samples were obtained (4 land-use types × 3 areas × 2 composite samples). After removing visible stones and roots, the samples were sieved through a 2 mm mesh. Fresh soil samples were immediately placed in ice boxes and transported to the laboratory. A portion of each soil sample was stored at 4 °C and −80 °C for DNA extraction and microbiological analysis, while the rest were air-dried for the assessment of physical and chemical properties.

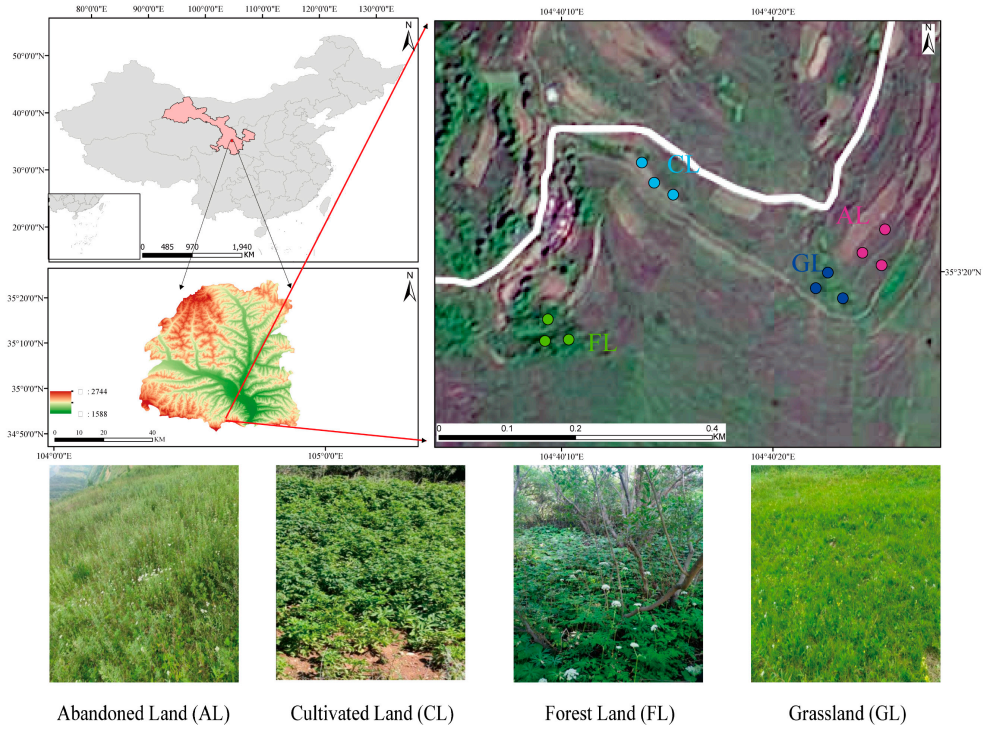


**Figure 1.** Location of the study area and experimental sites. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

#### 2.3.1. Determination of Soil Physical Properties

Soil water content was determined using the drying method. Soil pH was measured with a soil pH meter (PB-10, Sartorius, Göttingen, Germany) and bulk density (BD) was determined using the cutting ring method [30].

#### 2.3.2. Determination of Soil Chemical Properties and Enzyme Activities

Soil organic matter (SOM) was evaluated using potassium dichromate through external heating, and the total nitrogen (TN) content in the soil was determined using the Kjeldahl method. Ammonium nitrogen (AN) and nitrate nitrogen (NN) contents in soil samples were determined using KCl extraction and the indophenol blue colorimetric method. Soil alkaline nitrogen (SAN) was determined using NaOH alkaline hydrolysis in conjunction with the culture dish diffusion absorption method. Total phosphorus (TP) content in the soil samples was quantified using the molybdenum antimony resistance colorimetric method, while available phosphorus (AP) content was measured through potassium permanganate oxidation and glucose reduction. Furthermore, soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were determined using the chloroform fumigation and leaching method [30].

Soil sucrase (SUC) and soil cellulase (SCL) activities were assessed by employing a 3,5-dinitrosalicylic acid colorimetric assay, while urease activity (UER) was measured through a sodium phenol–sodium hypochlorite colorimetric assay. Similarly, alkaline phosphatase activity (ALP) in the soil was estimated using the disodium phenylphosphate colorimetric assay, while the assessment of catalase activity (CAT) was performed through potassium permanganate titration [31]. Furthermore, a soil polyphenol oxidase kit (Suzhou Keming Biotechnology Co., Suzhou, China) was used to analyze the activity of polyphenol oxidase (PPO) in soil samples.

### 2.4. Soil Quality Index (SQI) Assessment

In this study, 19 variables were selected, including both abiotic and biotic characteristics that varied significantly based on land use. The soil quality index (SQI) was calculated according to the methodology described previously [32]:

$$\mathrm{SQI} = \sum_{i=1}^{n} W_i \times S_i \quad (1)$$

where SQI represents the soil quality index, *Si* is the indicator score, *n* denotes the number of soil indicators, and *Wi* represents the weighting value assigned to each soil indicator.

### 2.5. DNA Extraction, Library Construction, and High-Throughput Sequencing

DNA was extracted from soil samples using the CTAB method. The levels of DNA degradation, potential contamination, and DNA concentration were assessed using an Agilent 5400 [33]. The soil macro-genome sequencing was performed at Wekemo Tech Group Co., Ltd., Shenzhen, China. The sequencing library was prepared using the NEBNext® UltraTM DNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA, Catalogue No: E7370L) following the manufacturer's guidelines, and index codes were assigned to each sample. Raw data for fungi, bacteria, and viruses in the soil samples were obtained through metagenomic sequencing using the Illumina Novaseq high-throughput sequencing platform.

### 2.6. Statistical Analysis

Data were organized through Microsoft Excel 2019. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was employed to assess significant differences ($p < 0.05$) in the soil properties and alpha diversity between four land-use types (SPSS 26.0, SPSS Inc., Chicago, IL, USA), the results of the measurements were expressed as means and standard errors. ArcGIS 10.2 was used to draw distribution maps for the plots across various land-use types, while Origin 2020 was employed to generate box plots illustrating the changes in soil bacterial and fungal diversity indices among the different land-use types. The OTU data were analyzed to draw histograms depicting the relative abundance of microorganisms across various taxonomic levels, and Venn diagrams illustrating the number of unique and shared OTUs among different land-use types. A linear discriminant analysis effect size (LEfSe) method was used to identify species displaying significant variation in abundance between the soil bacterial and fungal communities across various land-use types. Principal Coordinate Analysis (PCoA) based on Bray–Curtis distance was used to determine the β diversity of soil bacterial and fungal communities under different land-use types. The Wekemo cloud platform (www.bioincloud.tech, from 1 July 2024 to 15 October 2024) was used to construct the soil microbial network and analyze its parameters, while Gephi 0.10.0 software was employed to create co-occurrence maps of the soil microbial network across different land-use types. Redundancy analysis (RDA) was conducted to examine the relationships between soil properties and microbial communities across varying land-use types. Furthermore, the Spearman correlation matrix and Mantel test were used to assess the correlations between the relative abundances of soil bacterial and fungal communities and various environmental factors within these land-use types.

## 3. Results

### 3.1. Variations in SQI Across Four Land-Use Types and Influencing Factors

SWC, UER, and ALP varied significantly among the four land-use types. Specifically, soil samples from FL and GL demonstrated markedly higher levels of SWC, SOM, TN, AN, NN, and SAN compared to AL and CL (Table 1).

The dataset for calculating the SQI included 19 soil parameters that demonstrated significant differences (Table 1). The SQI rankings among the four land-use types were as follows: CL (0.25) < AL (0.30) < FL (0.52) < GL (0.54) (Figure 2).

**Table 1.** Soil parameters measured under different land-use types (mean ± standard deviation). Different lowercase letters in the rows represent significant differences at $p < 0.05$.

| Soil Characteristics | | Abandoned Land (AL) | Cultivated Land (CL) | Forest Land (FL) | Grassland (GL) |
|---|---|---|---|---|---|
| physical | SWC (%) | 11.88 ± 1.33 d | 16.62 ± 0.28 c | 24.17 ± 0.21 b | 27.99 ± 1.09 a |
| | pH | 7.62 ± 0.02 b | 8.13 ± 0.02 a | 7.29 ± 0.05 c | 7.56 ± 0.06 b |
| | BD ($g \cdot cm^{-3}$) | 1.33 ± 0.02 a | 1.41 ± 0.02 a | 1.01 ± 0.03 b | 1.04 ± 0.06 b |
| chemical | SOM ($g \cdot kg^{-1}$) | 4.44 ± 0.26 c | 5.85 ± 1.08 c | 15.07 ± 1.45 b | 19.07 ± 0.72 a |
| | TN ($g \cdot kg^{-1}$) | 1.22 ± 0.04 c | 1.05 ± 0.08 c | 1.79 ± 0.21 b | 2.67 ± 0.12 a |
| | AN ($mg \cdot kg^{-1}$) | 1.47 ± 0.22 c | 1.28 ± 0.05 c | 3.95 ± 0.67 b | 5.86 ± 1.01 a |
| | NN ($mg \cdot kg^{-1}$) | 2.62 ± 0.23 bc | 2.28 ± 0.08 c | 3.86 ± 0.46 b | 6.97 ± 0.70 a |
| | SAN ($mg \cdot kg^{-1}$) | 85.85 ± 3.65 b | 67.31 ± 9.38 b | 138.07 ± 16.58 a | 164.87 ± 9.43 a |
| | TP ($g \cdot kg^{-1}$) | 0.89 ± 0.01 ab | 0.84 ± 0.02 b | 0.60 ± 0.04 c | 0.94 ± 0.01 a |
| | AP ($mg \cdot kg^{-1}$) | 6.75 ± 0.04 b | 28.36 ± 0.75 a | 3.42 ± 0.13 c | 5.94 ± 0.04 b |
| | MBC ($mg \cdot kg^{-1}$) | 258.32 ± 12.57 c | 433.09 ± 26.09 b | 836.14 ± 57.85 a | 456.66 ± 46.39 b |
| | MBN ($mg \cdot kg^{-1}$) | 28.95 ± 0.43 c | 36.21 ± 1.07 b | 52.02 ± 3.91 a | 37.02 ± 1.48 b |
| | MBP ($mg \cdot kg^{-1}$) | 8.55 ± 0.09 b | 17.77 ± 0.56 a | 17.91 ± 1.05 a | 10.64 ± 0.92 b |
| Enzy | UER $mg/(g \cdot d^{-1})$ | 10.88 ± 0.18 b | 7.44 ± 0.17 d | 14.43 ± 0.08 a | 9.15 ± 0.06 c |
| | ALP $mg/(g \cdot d^{-1})$ | 83.68 ± 0.15 a | 51.02 ± 0.15 d | 80.24 ± 0.62 b | 79.04 ± 0.01 c |
| | CAT $mg/(g \cdot 30\ min^{-1})$ | 0.75 ± 0.02 b | 0.76 ± 0.03 b | 0.63 ± 0.01 b | 1.31 ± 0.11 a |
| | SCL $mg/(g \cdot d^{-1})$ | 0.12 ± 0.01 a | 0.02 ± 0.00 c | 0.08 ± 0.00 b | 0.12 ± 0.00 a |
| | SUC $mg/(g \cdot d^{-1})$ | 16.04 ± 0.50 a | 4.85 ± 0.75 c | 10.42 ± 1.11 b | 15.18 ± 0.84 a |
| | PPO $mg/(g \cdot d^{-1})$ | 16.54 ± 1.34 a | 5.87 ± 0.10 b | 17.41 ± 1.57 a | 6.98 ± 0.07 b |

Note: AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland; SWC, soil water content; BD, bulk density; SOM, soil organic matter; TN, total nitrogen; AN, ammonia nitrogen; NN, nitrate nitrogen; SAN, alkali-hydrolyzable nitrogen; TP, total phosphorus; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; UER, soil urease; ALP, soil alkaline phosphatase; CAT, soil catalase; SCL, soil cellulase; SUC, soil sucrase; PPO, soil polyphenol oxidase. Different letters show significant differences at $p < 0.05$.

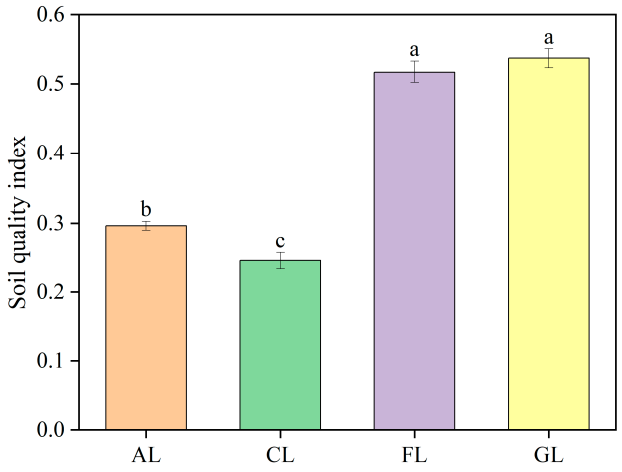


**Figure 2.** SQI values across various land-use types. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland. Different letters show significant differences at $p < 0.05$.

### *3.2. Variations in Soil Microbial Community Structure and Dominant Species Among Four Different Land-Use Types*

The abundance of soil bacterial and fungal communities was analyzed at both the phylum (Figure 3a,c) and genus levels (Figure 3b,d). The predominant bacterial phyla, Pseudomonadota and Actinomycetota, constituted over 50% of the soil bacterial communities across all four land-use types. The dominant bacterial genus within the Actinomycetota phylum was identified as *Bradyrhizobium*. However, the soil fungal communities were primarily composed of the Ascomycota, Mucoromycota, and Basidiomycota phyla, with the prevailing fungal genera being *Rhizophagus* and *Penicillium*.

A total of 13,076 microbial OTUs were identified in all soil samples, including 3037, 3905, 2983, and 3151 OTUs in the soils of AL, CL, FL, and GL, respectively. Moreover, there were 334, 848, 364, and 508 unique OTUs in AL, CL, FL, and GL, respectively, with a total

of 1629 common OTUs shared among the four land-use types (Figure 4a). Similarly, the total number of bacterial OTUs identified in the soil samples across the four land-use types was 11,815, comprising 2740 OTUs in AL, 3515 in CL, 2705 in FL, and 2855 in GL. Among these, there were 257, 709, 286, and 422 unique bacterial OTUs observed in the respective lands, with 1531 common OTUs (Figure 4b). Furthermore, the fungal OTUs identified in the four land-use types included 234 in AL, 286 in CL, 220 in FL, and 204 in GL, resulting in a total of 944 fungal OTUs. The unique fungal OTUs were 50 in AL, 77 in CL, 53 in FL, and 33 in GL, with 80 common OTUs shared among the four land-use types (Figure 4c).

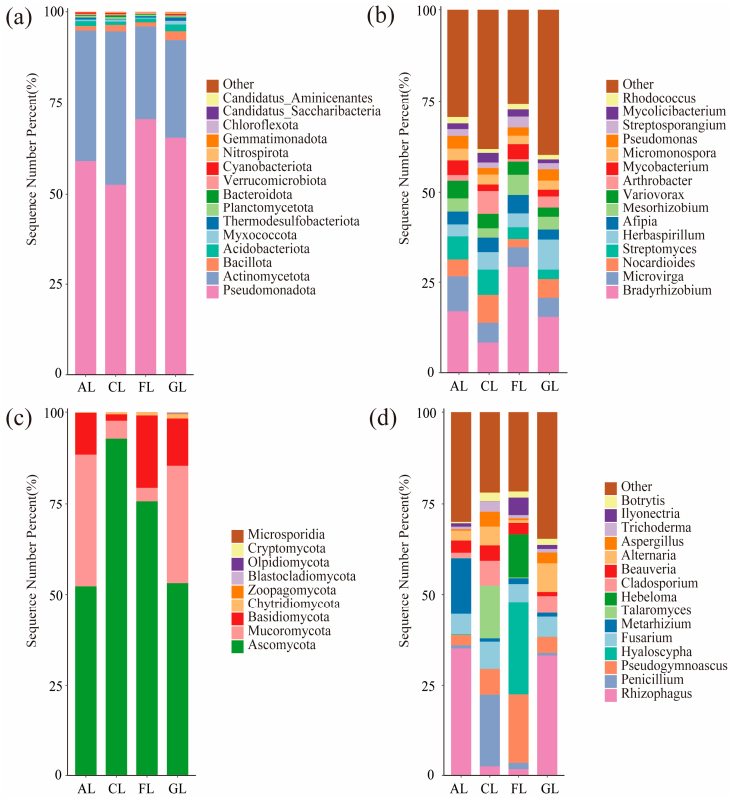


**Figure 3.** Relative abundance of soil bacteria at phylum (**a**) and genus levels (**b**). Relative abundance of soil fungi at phylum level (**c**) and genus level (**d**). AL: abandoned land; CL: cultivated land; FL: forest land; GL: grassland (feature = 15).

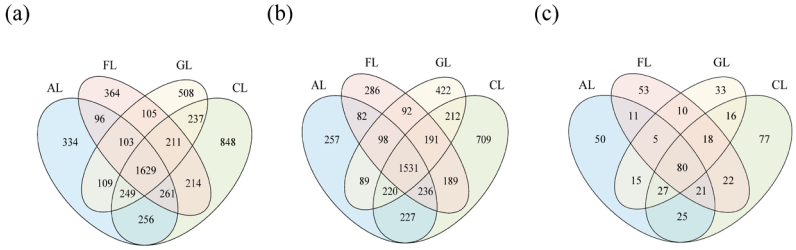


**Figure 4.** Venn diagram showing the OTUs of soil microbes (**a**), bacterial OTUs (**b**), and fungal OTUs (**c**) across various land-use types. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

Linear discriminant analysis (LDA) effect size (LEfse) was employed to identify the bacterial (Figure 5a) and fungal (Figure 5b) taxa showing significant differences in abundance across the four types of land. The results indicated significant differences in 37 bacterial and 36 fungal communities at an LDA threshold of 4.0. Pseudomonadota and Actinomycetota emerged as the dominant bacterial phyla, while Ascomycota, Basidiomycota, and Mucoromycota were identified as the predominant fungal phyla across the four land-use types. At the order level, Micrococcales, Hyphomicrobiales, and Burkholderiales were

the most significantly variable bacterial taxa identified in the soils of AL, FL, and GL, while Glomerales, Helotiales, Eurotiales, and Pleosporales emerged as the most significant discriminating fungal orders in the surface soils of AL, CL, FL, and GL.

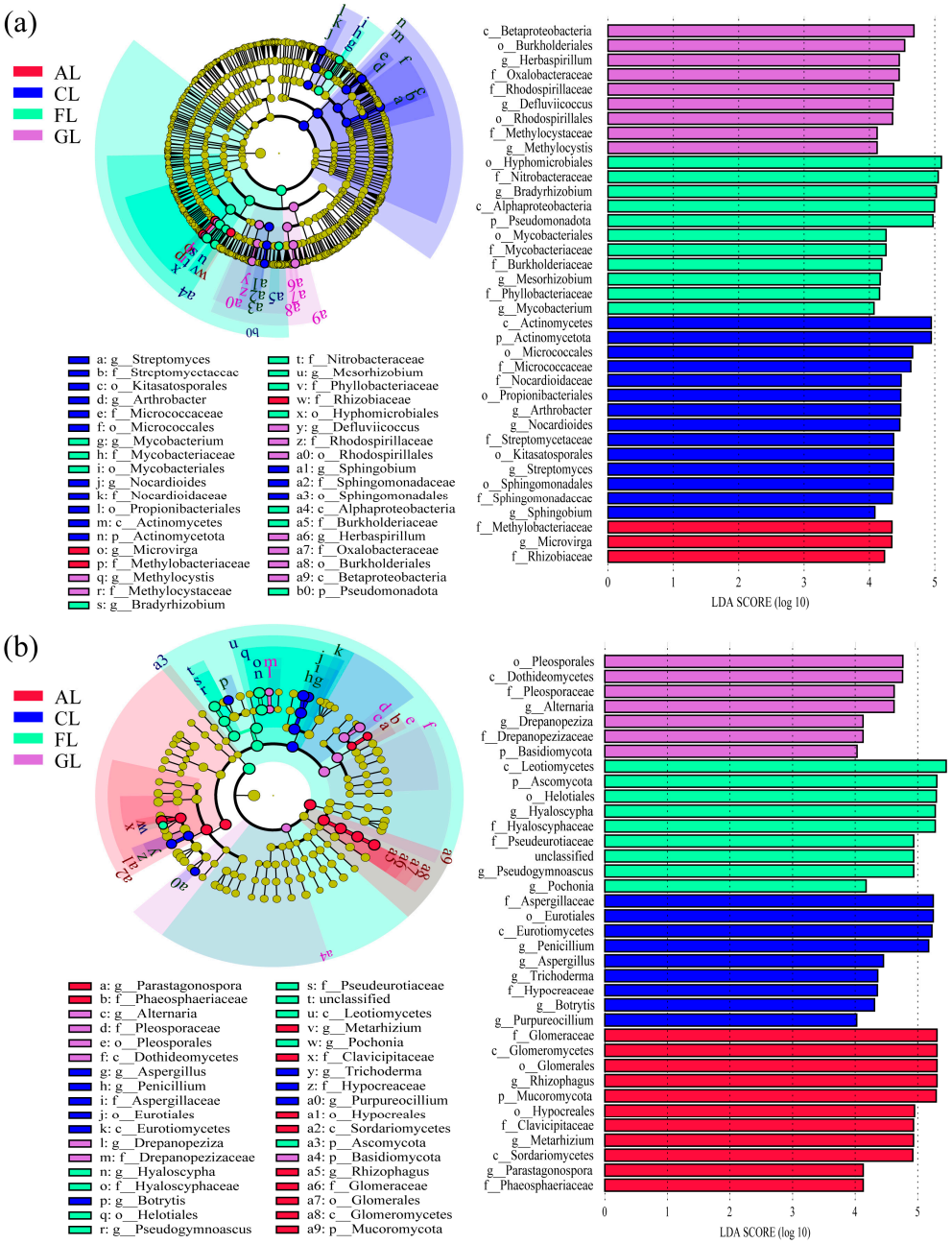


**Figure 5.** Differences in the compositions of soil bacterial (**a**) and fungal (**b**) communities under different land-use types were analyzed using linear discriminant analysis effect size (LEfSe). Each small circle at a classification level represents a taxon, with the diameter indicating the relative abundance of that taxon. Species with no significant differences are uniformly colored in yellow, while those with significant differences are represented by colored biomarkers. The names of species associated with the biomarkers are displayed on the right side, with letters and numbers corresponding to the figure. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

Box plots were used to illustrate the variations in the diversity indices of bacterial (Figure 6a) and fungal (Figure 6b) communities under different land-use types. The diversity indices of both bacterial and fungal communities displayed a consistent trend among the four land-use types. In terms of the Chao1 index versus ACE index, bacterial and

fungal communities in CL demonstrated significant differences compared to those in AL, FL, and GL, while no significant differences were observed among the communities of AL, FL, and GL. Conversely, the Shannon and Simpson indices for both bacterial and fungal communities in CL were significantly higher than those in AL and FL, with GL showing the lowest Shannon and Simpson indices.

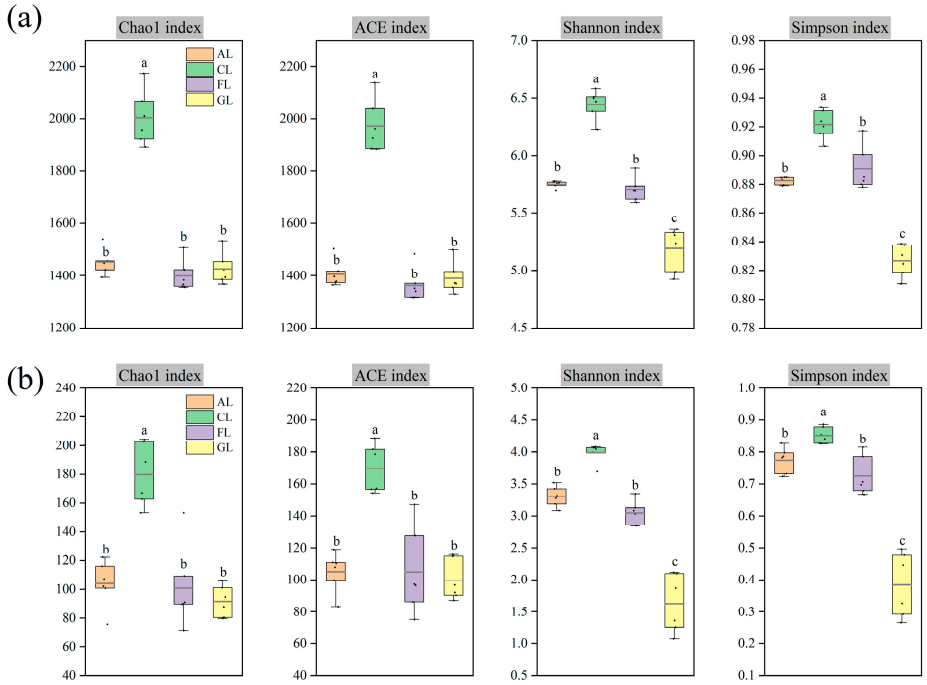


**Figure 6.** Soil bacterial (**a**) and fungal (**b**) community diversity indexes across different land-use patterns. Letters indicate significant differences in diversity ($p < 0.05$). AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

PCoA analysis based on Bray–Curtis distance (Figure 7) showed different clusters of bacterial and fungal communities across the four lands. For the bacterial community (Figure 7a), contributions of PCoA1 and PCoA2 were found to be 34.99% and 25.41%, respectively. A denser distribution was observed for the six sampling sites of AL, indicating minimal differences between the bacterial communities at these sites. Conversely, for the fungal community (Figure 7b), PCoA1 and PCoA2 accounted for 33.2% and 19.13% of the variation, respectively. Similarly, the six sampling sites in GL demonstrated a denser distribution, suggesting minimal differences among the fungal communities at these locations.

Correlation networks of soil bacterial (Figure 8a) and fungal (Figure 8b) communities were constructed to better understand the microbial interactions across the four different land-use types. The phylum Actinomycetota emerged as a common bacterial phylum among all land-use types, while Ascomycota, Basidiomycota, and Mucoromycota were identified as the prevalent fungal phyla. Subsequently, the composition and topology of soil bacterial and fungal communities across the four land-use types were compared (Table 2), including the number of nodes and edges, average degree, network diameter, modularity index, and average clustering coefficient. The modularity index exceeding 0.4 suggested that the network had a modular structure. The results revealed positive correlations among the nodes representing microbial communities across all four land-use types. The community networks of FL and GL demonstrated a greater number of edges and a higher average degree compared to those of AL and CL, although their modularity coefficients were lower. Furthermore, the microbial networks in FL and GL were more intricate than those found in AL and CL. Notably, AL had the highest modularity index, signifying a more distinct structure in its soil microbial community.

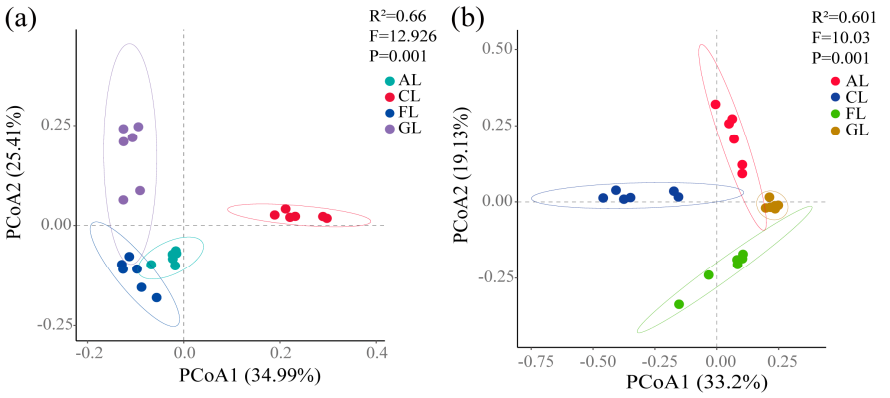


**Figure 7.** Principal component analysis (PCoA) of bacterial (**a**) and fungal (**b**) communities in different land-use types. Nodes indicate the OTUs, with distinct modules displayed in various colors. The red line represents a positive correlation, while the green line denotes a negative correlation. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

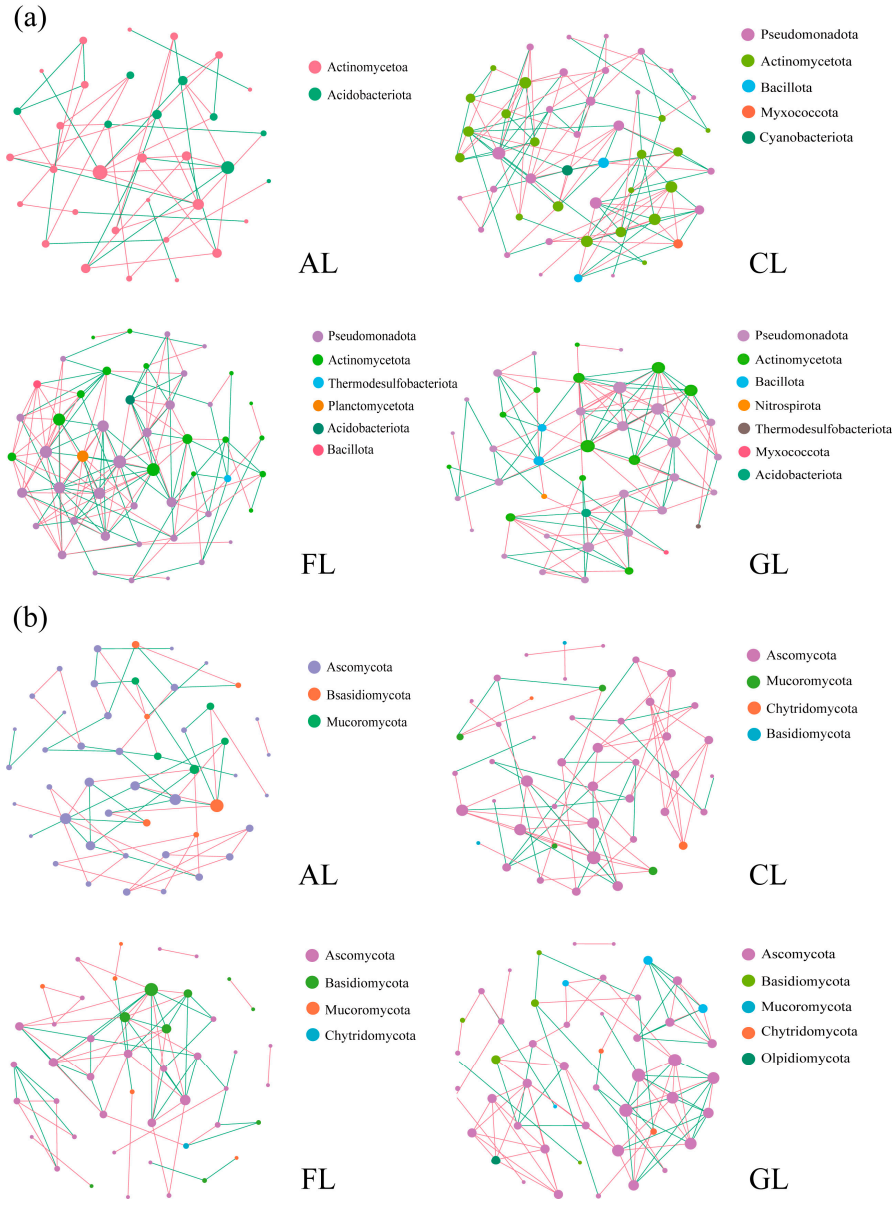


**Figure 8.** Co-occurrence networks of soil bacterial (**a**) and fungal (**b**) communities across different land-use types. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

**Table 2.** Topological properties of the co-occurrence networks of the bacterial and fungal community in soils of different land-use types.

| | | AL | CL | FL | GL |
|---|---|---|---|---|---|
| Bacterial | Nodes | 34 | 48 | 48 | 44 |
| | Links | 49 | 116 | 156 | 126 |
| | Average degree | 2.882 | 4.833 | 6.5 | 5.727 |
| | Network diameter | 6 | 9 | 10 | 9 |
| | Modularity | 0.725 | 0.674 | 0.503 | 0.529 |
| | Average clustering coefficient | 0.687 | 0.642 | 0.583 | 0.603 |
| | Average path length | 2.446 | 3.994 | 3.419 | 3.341 |
| | Positive correlation connection | 61.22% | 50.86% | 51.92% | 53.97% |
| | Negative correlation connection | 38.18% | 49.16% | 48.08% | 46.03% |
| Fungi | Nodes | 48 | 46 | 46 | 49 |
| | Links | 61 | 73 | 74 | 97 |
| | Average degree | 2.542 | 3.174 | 3.217 | 3.959 |
| | Network diameter | 10 | 12 | 8 | 9 |
| | Modularity | 0.771 | 0.707 | 0.584 | 0.723 |
| | Average clustering coefficient | 0.545 | 0.514 | 0.577 | 0.646 |
| | Average path length | 4.388 | 4.513 | 2.566 | 3.303 |
| | Positive correlation connection | 52.46% | 65.75% | 52.7% | 60.82% |
| | Negative correlation connection | 47.54% | 34.25% | 47.3% | 39.18% |

Note: AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland.

### *3.3. Relationships Between Soil Properties and Microbial Communities Under Four Different Land-Use Types*

RDA of soil physicochemical properties and microbial communities (Figure 9) indicated significant variations in community composition across the various land-use types. In total, soil properties accounted for 95.13% of the variability observed in the soil bacterial community, with the RDA 1 and RDA 2 axes of the RDA explaining 71.84% and 23.29% of this variation, respectively. Similarly, soil properties could explain 80.80% of variations in the soil fungal community, with RDA 1 axis and RDA 2 axis explaining 70.20% and 9.88% of variations, respectively. Furthermore, soil bacterial communities of AL were primarily influenced by SWC, SOM, AN, NN, and TN, while its fungal communities were primarily affected by MBC. In CL, bacterial communities were predominantly influenced by SWC, SOM, AN, NN, SAN, and TN, while fungal communities were primarily affected by SWC, SOM, AN, SAN, and NN. In FL, TP emerged as the primary factor influencing the bacterial communities, while fungal communities were influenced by TP, BD, and pH. Similarly, TP was the key factor influencing the bacterial communities in GL, while BD, pH, and AP significantly impacted the fungal communities. Furthermore, the dominant bacterial phylum Pseudomonadota was primarily affected by TP, while Actinomycetota was influenced by several factors, including SWC, SOM, AN, SAN, NN, and TN. SWC and AP accounted for 60.7% and 18.8% of the variations in the bacterial communities, respectively. On the other hand, the dominant fungal phylum Ascomycota was primarily affected by SWC, SOM, SAN, AN, TN, and NN, while Mucoromycota was predominantly influenced by SWC. TP was identified as the primary factor affecting the abundance of Basidiomycota. Furthermore, TN and TP accounted for 47.8% and 15.3% of the variations observed in the fungal community, respectively.

Mantel's experiment explored the relationships between microbial diversity and soil properties across the four different land-use types. The results showed that both bacterial and fungal diversity indices were affected by soil pH, SAN, AP, UER, and ALP (Figure 10).

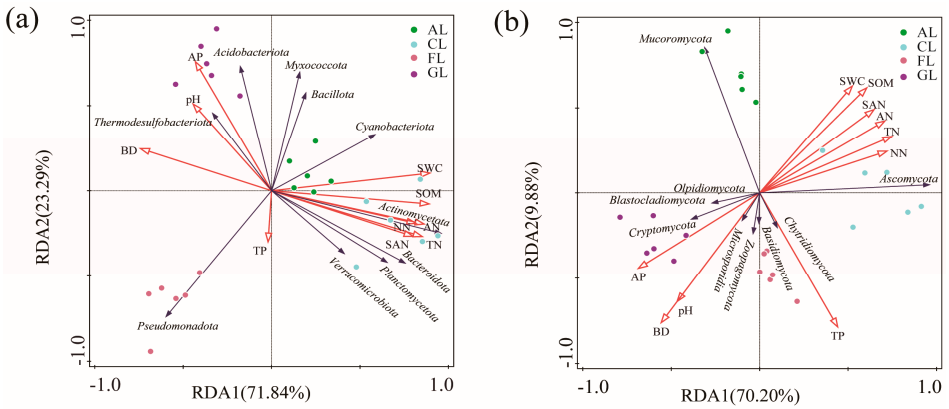


**Figure 9.** Redundancy analysis (RDA) of bacterial (**a**) and fungal (**b**) communities and physicochemical properties of soil across different land-use types. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland; SWC, soil water content; BD, bulk density; SOM, soil organic matter; TN, total nitrogen; AN, ammonia nitrogen; NN, nitrate nitrogen; SAN, alkali-hydrolyzable nitrogen; TP, total phosphorus; AP, available phosphorus.

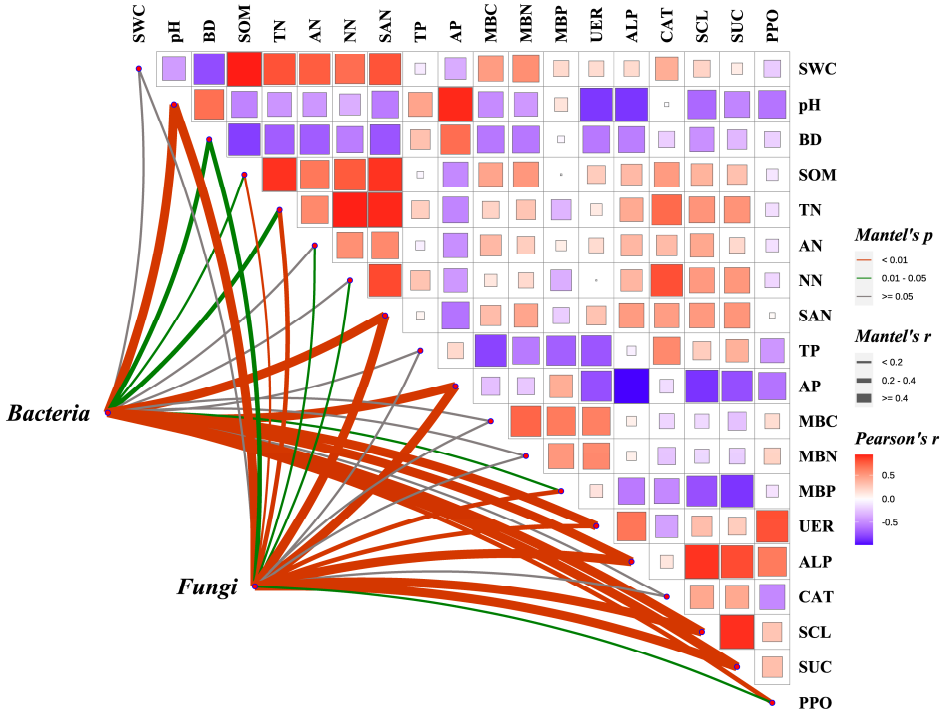


**Figure 10.** Mantel test showing the correlations between soil factors and microbial diversity. The width of the edges represents the absolute value of the correlation coefficients derived from the Mantel test. Colors show the strength of significant correlations. Pairwise comparisons of soil factors are displayed in rectangles, with color gradients demonstrating Pearson's correlation coefficients. AL, abandoned land; CL, cultivated land; FL, forest land; GL, grassland; SWC, soil water content; BD, bulk density; SOM, soil organic matter; TN, total nitrogen; AN, ammonia nitrogen; NN, nitrate nitrogen; SAN, alkali-hydrolyzable nitrogen; TP, total phosphorus; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; UER, soil urease; ALP, soil alkaline phosphatase; CAT, soil catalase; SCL, soil cellulase; SUC, soil sucrase; PPO, soil polyphenol oxidase.

## 4. Discussion

### *4.1. Changes in Soil Properties and SQI Across Various Land-Use Types*

In this study, the lowest soil pH was observed in FL for the same soil background, likely due to the acidifying effects of root mucilage and exudates [34], as well as the decomposition

of leaves. Furthermore, AL, FL, and GL showed higher plant diversity and surface litter than CL, potentially contributing to the development of more stable aggregate structures and carbon pools [35]. The described enhancement may positively influence soil structure stabilization and the overall improvement of soil physical properties [36]. Simultaneously, FL and GL facilitated the accumulation of soil carbon and nitrogen; however, phosphorus levels in these areas were determined to be lower than those in CL (Table 1), which can be attributed to fertilizer application in the soil of CL. The changes in land-use patterns lead to variations in physical structure, hydrothermal conditions, and nutrient levels of soils, which further affect the soil enzyme activities and biochemical cycling processes within terrestrial ecosystems [37]. This study found that compared to the other three land types, CL had lower soil enzyme activity, indicating that human interference activities such as tillage and fertilization had a negative impact on soil enzyme activity and led to the depletion of soil nutrients. Furthermore, higher soil enzyme activities in GL, FL, and AL can be attributed to a higher quantity of aboveground plant roots and apomictic material, providing a nutrient-rich environment that enhances soil health and fertility.

The transformation of land from CL to AL, FL, and GL in the Longzhong Loess Plateau altered the soil's physical and chemical properties, resulting in a prominent increase in SQI (Figure 2). Traditional cultivation practices decreased soil fertility due to anthropogenic disturbances, including the breakdown of soil aggregates, harvesting of plant biomass, and removal of surface litter [38]. The described phenomenon explains the lowest SQI observed in CL in this study; this corresponds to our hypothesis. On the other hand, SQI in AL was significantly higher than that in CL, likely due to reduced anthropogenic disturbances and increased surface litter accumulation during natural restoration [39]. The higher SWC in GL and FL, beneficial for plant growth, can be attributed to dense aboveground plant cover, reduced surface transpiration, low water consumption by plants, and neutral soil pH conditions in these lands. Furthermore, lower soil BD contributes to a looser and more porous soil structure, therefore improving the aeration and permeability of soil, which further improves the SQI.

### *4.2. Effects of Various Land-Use Types on Soil Microbial Communities*

The structure of soil microbial communities was determined by land-use patterns and the physicochemical properties of soil [40]. Consistent with earlier research on soil microbial communities in the Loess Plateau region [41], the predominant bacterial phyla identified in this study were Pseudomonadota and Actinomycetota. Pseudomonadota is an essential bacterial group that significantly contributes to organic matter decomposition and carbon cycling [42]. In this study (Figure 3), the relative abundance of Pseudomonadota in FL was higher compared to the other three land-use types. The most abundant order within Pseudomonadota was Rhizobiales, with *Bradyrhizobium* being the dominant genus across all four land-use types, particularly prevalent in the bacterial communities of FL soils. *Bradyrhizobium* represented over 25% of the total bacterial genera, highlighting that nitrogen-cycling bacteria are crucial components of the microbial community in the arid soils of the Longzhong Loess Plateau. Actinomycetota are typically recognized as spore-forming bacteria, frequently thriving in nutrient-poor and stressed soil environments [43]. In this study, the relative abundance of Actinomycetota in CL was higher than those in the other three land-use types, aligning with the observation of the lowest SQI in CL; this result is consistent with our second hypothesis. The dominant fungal phyla were Ascomycota, Mucoromycota, and Bsaidiomycota, consistent with previous research [44]. In particular, the abundance of Mucoromycota was higher in AL and GL compared to CL and FL. Based on their phylogenetic characteristics, members of Mucoromycota can form either beneficial or pathogenic relationships with green hosts [45]. Fungi belonging to the Basidiomycota and Ascomycota phyla serve as the primary decomposers in soil ecosystems, typically comprising more than 90% of the microbial community responsible for decomposition [46]. The relative abundances of Basidiomycota were significantly higher in the soils of AL, FL, and GL compared to CL. This observation indicates that vegetation cover likely supports the

establishment of more stable Basidiomycota communities, whereas agricultural activities can disrupt their mycelial networks [47].

Venn diagrams of shared OTU data, along with microbial branches identified through LEfSe analyses and PCoA analyses using Bray–Curtis distances, demonstrated a distinct separation in the bacterial and fungal community composition of CL compared to AL, FL, and GL. The distinct microbial co-occurrence networks observed across the four land-use types due to the unique interactions within soil habitats. These differences provided valuable insights into potential microbial interactions, relationships, functions, and ecological roles specific to each land-use type [48]. Nodes and edges in a microbial network represent microbial abundance and interspecies correlations, respectively. The number of nodes and edges, along with the average degree, reflect the size and complexity of the network, whereas a higher average degree indicates more complex connections between nodes [49]. In this study, the microbial co-occurrence networks of FL and GL demonstrated higher nodes, edges, and a higher average degree, indicating larger microbial (bacterial and fungal) networks and more complex interactions among microbial species in these lands compared to CL and AL. Positive correlations dominated the soil bacterial and fungal networks across all four land-use types, suggesting that synergistic cooperation and minimal competition between soil fungi and bacteria were prevalent in each land-use type. Furthermore, bacterial and fungal networks of AL, FL, and GL showed shorter average path lengths compared to CL, indicating a more efficient transfer of material, energy, and information between the microbial species in these three lands. This finding corresponded to the higher SQI of AL, FL, and GL compared to CL. Furthermore, abundance (ACE and Chao1) and diversity (Shannon and Simpson) indices of bacterial and fungal communities were significantly influenced by variations in land-use type. Compared to bacterial communities, fungal communities displayed lower diversity and were less dependent on soil chemistry. The abundance and diversity of soil bacterial and fungal communities in CL were significantly higher than those in the other three land-use types. The lowest bacterial and fungal diversities were observed in GL, which was consistent with the previous findings [50]. However, soil bacterial and fungal communities demonstrated significantly higher diversity in the forested areas of the Loess Plateau compared to cultivated regions, likely due to substantial human disturbance in the cultivated areas [51]. Long-term cultivation and fertilization, as well as other field management measures, can alter soil porosity and provide additional energy sources for microbes [52], which in turn promote microbial growth and enhance both diversity and abundance. These findings support the hypothesis that soil microbial communities are influenced by varying land-use patterns and human activities.

### *4.3. Response of Microbial Communities to Soil Physicochemical Properties Across Different Land-Use Types*

In this study, RDA showed the correlations between microbial communities and physicochemical properties of soil under the four land-use types. Various previous studies have demonstrated that soil physicochemical factors significantly influence microbial communities [53]. Different bacterial species often require a specific pH range for optimal growth, which may exert challenges for some microbes when competing for resources [54]. Although the four types of land use have similar soil backgrounds, the changes in land-use types caused by human interference are more pronounced in the soil properties of the Loess Plateau. The CL bacterial community was primarily influenced by the content of nitrogen. The abundance of Actinomycetota was higher in CL and showed a positive correlation with the levels of Nitrogen element, SWC, and SOM. The observed finding was consistent with previous research [55], which indicated that Actinomycetota abundance increased with higher nitrogen content. However, it remains uncertain whether Actinomycetota belongs to the eutrophic or oligotrophic group [41]. However, the lower Actinomycetota abundance in the other three land-use types, along with the SQI value in CL, suggested that Actinomycetota tended to favor oligotrophic conditions in this study area. The bacterial community in AL displayed similar behavior to that of CL. Moreover, the abundance of

Pseudomonadota was higher in FL compared to the other three land-use types, showing a positive correlation with the concentrations of TP in the soil. According to a previous study, these bacteria prefer eutrophic soil environments [56]. Pseudomonadota, as the dominant bacterial phylum, plays a vital role in the soil ecosystems of arid and semi-arid regions. Soil bacterial communities in GL were primarily influenced by TP. The variations in soil bacterial communities at the phylum level under the four land-use types can be attributed to the distinct nutrient utilization strategies employed by the microbes [57]. The soil microorganisms can be classified into two ecological classes based on the co-nutrient (r)-oligotrophic (k) framework [58]. In this study, the soil bacterial community shifted from the k-strategy to r-strategy along with the SQI gradient. The finding was consistent with the results reported previously [59].

The distribution of fungal communities is usually correlated with the phosphorus content in soil. In this study, the abundance of Basidiomycota increased with the increase in soil phosphorus content. Furthermore, Basidiomycota predominated in FL, and the fungal communities in the soil of FL displayed a positive relationship with AP content. The Basidiomycota phylum primarily comprised exogenous mycorrhizal fungal species that promote afforestation [40]. A higher abundance of Ascomycota has been reported in phosphorus-rich soils compared with Basidiomycota [60]. In this study, the phosphorus content was higher in CL soil, which accounted for the higher relative abundance of Ascomycota in this area. Generally, SWC predominantly accounted for the variations in soil microbial communities, likely due to its relationship with the litter content in the soil. The litter content in FL and GL was higher than that in CL, contributing to a reduction in soil water evaporation. Simultaneously, litter provided a good habitat for microbial communities. Furthermore, soil pH may have a direct or indirect effect on microbial $\alpha$-diversity [61]. The Mantel analysis of soil physicochemical properties and microbial $\alpha$-diversity across the four land-use types demonstrated significant correlations between bacterial and fungal $\alpha$-diversity and factors including pH, SAN, MBC, UER, ALP, SCL, and SUC. Soil texture significantly influenced the $\alpha$-diversity of the soil microbial community, while SOM, TN, and TP had less pronounced effects. Soil texture positively affected the soil's chemical properties. Furthermore, SWC was observed to be positively correlated with SOM, TN, AN, NN, and SAN, while pH and BD demonstrated positive correlations with TP and AP. Similarly to the findings of [62], the altered physicochemical properties of soil under different land-use types were the primary factors influencing the structure and diversity of soil microbial communities. Overall, SWC emerged as the key factor affecting the soil microbial communities in the Longzhong Loess Plateau region.

However, this study examined the influence of land-use patterns on soil microbial communities in only one area, rather than across multiple locations. The small-scale and single-time approach may lead to uncertainties and unexplained variations in the soil microbial communities. To better understand seasonal variations, further research is required in similar areas, allowing for broader and more reliable conclusions based on the larger sample size. Furthermore, future studies should consider other factors, such as aboveground plant diversity and soil fauna, to gain a deeper understanding of the effects of varying land-use patterns on the microbial communities and physicochemical properties of soil in the Loess Plateau.

## 5. Conclusions

Land-use types significantly affected the physicochemical properties of soils in the Longzhong region of the Loess Plateau. The results showed that compared with other land-use types, cultivated land had the lowest SQI value, but the diversity of soil bacterial and fungal communities in cultivated land was significantly higher than that of other land-use types. The relative abundance of Pseudomonadota in cultivated soil is relatively low, while the relative abundance of Actinomycota is relatively high. The relative abundance of Ascomycota in cultivated land fungal communities is significantly higher than that of other land-use types. The symbiotic network of soil microorganisms in cultivated

land is close to FL and GL, and human cultivation activities promote the interaction between soil microorganisms. Compared with other land-use methods, farmland has reduced aboveground cover and soil nutrients due to frequent farming activities and crop harvesting. SWC, SOM, SAN, TN, and NN all have an impact on soil bacterial and fungal communities in farmland. The diversity of soil bacterial and fungal communities is mainly influenced by pH, NN, and AP. Overall, in contrast to the other three different land-use patterns, the findings emphasized the influence of frequent human activities on soil quality and microbial communities in cultivated land. This study offers valuable insights regarding the effects of environmental factors on soil microbial communities.

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