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Analysis of Changes in Herbaceous Peony Growth and Soil Microbial Diversity in Different Growing and Replanting Years Based on High-Throughput Sequencing

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Abstract: The herbaceous peony (*Paeonia lactiflora* Pall.), a perennial herbaceous flower, can grows continuously for approximately 10 years. However, a replanting problem can occur during division propagation which reduces the land use rate and restricts the development of the herbaceous peony industry. We investigated microbial community changes and soil chemical properties in herbaceous peony soils during different growing and replanting years. The results indicated that the flowering rate, plant height, stem diameter, and leaf area of replanted herbaceous peony were lower, and decreased gradually with increasing replanting years. Compared with the soil after replanting herbaceous peony for one year, soil pH, nutrient contents (AN, AP, AK, and OM), enzyme activities (Inv, Ure, Pho, and Cat), diversity and richness of fungal and bacterial communities decreased after replanting for five years. Long-term replanting increased the relative abundance of harmful soil microorganisms (e.g., *Gibberella*), and reduced that of beneficial microorganisms (e.g., *Bacillus*). Overall, after the long-term replanting of herbaceous peony, the soil environment deteriorated, and the soil microbial community structure changed, resulting in the imbalance of soil microecology, damaging the normal growth of herbaceous peony.

Keywords: *Paeonia lactiflora* Pall.; microbial diversity; replanting; continuous growth; high-throughput sequencing

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1. Introduction

The herbaceous peony (*Paeonia lactiflora* Pall.) is a world-famous flower belonging to the family Paeoniaceae and it. The herbaceous peony has large, colorful flowers and high ornamental and medicinal value. The international flower market has widely sought it as a fresh cut flower. Therefore, the market demand for high-quality herbaceous peony seedlings and cut flowers has considerably increased. During herbaceous peony production, division propagation is predominantly undertaken. The mother peony is divided vertically for planting from the root cap into several daughter plants with several buds. This method is easily undertaken and can maintain varietal characteristics. To expand seedling breeding, ramet seedlings can be divided again after being grown for over three years, after which fresh cut flowers can be harvested annually [1]. If healthy plants have been grown for ten years with sufficient space, they can also be used for cut flower harvesting [2]. If ramet seedlings are planted at the original planting site, the growth and flowering rates are reduced, with deteriorating quality and aggravation of pests and diseases, eventually resulting in plant mortality [3,4]. This reduces productive land use, increases production costs, restricts the promotion of fine varieties, and sustainable development of the industry.

Replanting problems exist in many plants, such as apples [5], lilies [6], grapes [7]. Soil microorganisms participate in energy flows and material cycling in the ecosystem and play

a key role in the maintenance of ecosystem stability [8]. In addition, soil microorganisms significantly influence agricultural soil productivity, plant growth, and crop quality [9]. Changes in the soil microbial community are a key contributing factor to problems derived from replanting [10,11].

Hua, et al. [12] found that harmful soil fungi accumulated and the presence of beneficial fungi was reduced after long-term replanting of lily, thus affecting the normal growth. Soil microbial diversity is directly proportional to plant productivity [13]. Bacteria and fungi diversity in soils with long-term replanting with species such as *Vitis vinifera* and *Rehmannia glutinosa* substantially decreased, leading to the decline of their quality and yield and the problem of replanting [14,15]. Li, et al. [16] found that after replanting *Pseudostellaria heterophylla*, soil nutrient content, soil enzyme activity, and beneficial bacteria decreased, and harmful bacteria increased, thus inhibiting the growth of *Pseudostellaria heterophylla*. Studying the soil microbial diversity of herbaceous peony to explore the mechanisms of the replanting problem should be a key research focus.

High-throughput sequencing technology (HTS) is widely used in the study of microbial community diversity, considering its rapid processing and high level of accuracy. It can correctly reflect the community structure of the rhizosphere microorganisms [17]. Xie, Sun, Zhang, Li, Liu, Li and Sun [4] found that the number of bacteria and fungi substantially decreased and increased, respectively, with increasing years of replanting of herbaceous peony. However, the change in microbial diversity in herbaceous peony soil under replanting conditions has not been reported. Therefore, we studied soil microbial community composition changes encompassing different growing and replanting years. We analyzed the relationship between plants, soil environmental factors, and microbial communities in herbaceous peony to find the key microbial flora that affect plant growth. This study can provide a theoretical reference for exploring the mechanisms of herbaceous peony replanting problems and promote the development of the herbaceous peony industry.

2. Materials and Methods

2.1. Site Description and Sampling Selection

The experiment was undertaken at the Peony Resource Nursery and Horticultural Experiment Center of the Horticultural Experiment Station of Shandong Agricultural University (Tai'an City, Shandong Province of China; 35°38′–36°28′ N and 116°20′–117°59′ E). Six 'Hongfengyu' herbaceous peony varieties from different growing and replanting years from three plantations were selected for the study. This included plants grown for one year in a 1-year plantation (Z1-1); plants grown continuously for five years in a 5-year plantation (Z5-5); plants replanted for one year in a 5-year plantation (H5-1); plants grown continuously for eight years in an 8-year plantation (Z8-8); plants replanted for one year in an 8-year plantation (H8-1); and plants replanted for five years in an 8-year plantation (H8-5). Bulk soils (CK1, CK5, CK8) were collected from the middle of two rows of plants from each plantation as the control (Figure S1). Division propagation was carried out yearly in October, with each plant retaining five scale buds, and the planting density was 80 × 80 cm. The cultivation and management measures implemented remained consistent throughout the study.

On 14 April 2021, three plants of each type were randomly selected to collect rhizosphere soil. Briefly, the whole plant was dug up, shaken and large chunks of soil were removed from the roots, and the soil was collected 2 mm from the surface of the root. Three bulk soils (the same depth as the root of plants) were randomly collected from each plantation. Soil samples were immediately transported to the laboratory where they were sieved (Φ = 1 mm) and divided into two parts. One part was stored at −80 °C for DNA extraction, whereas the other was air-dried to determine the physical and chemical properties. The excavated plants were used to determine plant morphological indices and fibrous roots were used to measure root activity.

2.2. Determination of Plant Morphological Indices Root Activity

A tape measure was used to obtain the height from ground level to the heart leaf on the main stem. Stem diameter was measured using Vernier calipers and leaf area was measured using a leaf area meter (TOP Cloud-agri, Zhuji, China). The flowering rate was calculated as the ratio of flowering branches to the number of main branches [18]. Root activity was measured using the triphenyl tetrazole chloride (TTC) method [19].

2.3. Determination of Soil Physicochemical Properties

Soil pH was measured using a PHS-2F pH meter (LEICI, Shanghai, China) and soil electrical conductivity (EC) was measured using a DDB-303A portable conductivity meter (LEICI, Shanghai, China). Alkali-hydrolyzable nitrogen (AN) was measured using the alkali hydrolysis diffusion method. Available phosphorus (AP) was measured using the sodium bicarbonate method. Available potassium (AK) was measured using the flame photometry method, and organic matter (OM) was measured using the potassium dichromate method. These analyses were based on the methods by Bao [20].

2.4. Determination of Soil Enzyme Activities

Soil invertase activity (Inv) was measured using colorimetry, soil urease activity (Ure) was measured using phenol sodium–sodium hypochlorite colorimetry, soil phosphatase activity (Pho) was measured using the disodium phenyl phosphate colorimetric method, and soil catalase activity (Cat) was measured using permanganate titration. These analyses were based on the methods by Guan [21].

2.5. DNA Extraction and PCR Amplification

The FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) was used for total DNA extraction according to the manufacturer's instructions. DNA concentration and purity were determined using a NanoDrop2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [22]. ITS regions of the fungal rRNA gene were amplified using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) [23]. PCR was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) with the following protocol: 3 min of initial denaturation at 95 °C; 27 cycles of denaturation for 30 s at 95 °C; annealing for 30 s at 55 °C; extension for 45 s at 72 °C; and a final step for 5 min at 72 °C. PCR reactions were performed in triplicate.

2.6. Illumina Miseq Sequencing and Data Processing

The PCR product was extracted from 2% agarose gel and purified using the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw sequencing reads were demultiplexed, quality-filtered by Fastp (version 0.19.6), and merged using FLASH (version 1.2.11) [24,25]. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using the UPARSE-OTU algorithm of USEARCH (version 7.0.1090), and chimeric sequences were identified and removed [26,27]. The OTU representative sequences used were identified in the SILVA database (version 138) and the UNITE database (version 8.0) using the RDP Classifier algorithm of QIIME (version 1.9.1), with a confidence threshold of 70% [28]. The OTUs data were rarefied to the minimum sample count for subsequent analysis, including alpha diversities and species Venn diagrams, community composition, and distance-based redundancy analysis (RDA).

2.7. Statistical Analysis

Rarefaction and alpha diversity indices (Shannon, ACE, Chao1, and Coverage) for each sample were calculated using Mothur (version v.1.30.2). Species Venn diagrams, community composition, and distance-based redundancy analysis (RDA) were performed using R software (version 3.3.1). These analyses were performed online using the Majorbio Cloud Platform. One-way ANOVA was conducted on herbaceous peony morphological indices, soil physicochemical properties, and microbial α-diversity using SPSS Statistics 26.0 (IBM Corporation, New York, NY, USA), followed by Duncan's multiple range test, and Microsoft Excel 2010 was used to construct the histograms.

3. Results

3.1. Changes in Morphological Indices

Table 1 showed that the flowering rate, plant height, stem diameter, and leaf area of Z5-5 increased by 125.11%, 25.14%, 26.11%, and 109.52%, respectively, compared with those of Z1-1. The flowering rate, stem diameter, and leaf area of Z8-8 increased by 57.37%, 11.46%, and 88.52%, respectively, and the plant height decreased by 5.69%, compared with those of Z1-1. The flowering rate, plant height, stem diameter, and leaf area of H5-1 and H8-1 were slightly lower than those of Z1-1. The flowering rate, plant height, stem diameter, and leaf area of H8-5 decreased significantly by 75.72%, 49.20%, 36.36%, and 52.41%, respectively, compared with Z5-5. The flowering rate, plant height, and stem diameter of H8-5 decreased by 44.33%, 20.83%, and 9.84%, respectively, compared with those of H8-1.

Table 1. Morphological indices of herbaceous peony under different planting and replanting years.

Sample		Flowering Rate (%)	Plant Height (cm)	Stem Diameter (cm)	Leaf Area (cm^2)
1-year plantation	Z1-1	43.33% ± 37.34% ab	56.20 ± 9.17 b	6.28 ± 0.97 bc	10.19 ± 4.11 b
5-year plantation	Z5-5	80.05% ± 20.89% a	70.33 ± 12.76 a	7.92 ± 0.40 a	21.35 ± 2.16 a
	H5-1	42.22% ± 36.72% ab	51.90 ± 1.64 b	6.15 ± 0.16 bc	7.12 ± 2.38 b
8-year plantation	Z8-8	55.96% ± 5.27% ab	53.00 ± 3.00 b	7.00 ± 0.42 ab	19.21 ± 8.82 a
	H8-1	34.92% ± 7.27% ab	45.13 ± 4.83 bc	5.59 ± 0.09 c	7.25 ± 0.68 b
	H8-5	19.44% ± 17.35% b	35.73 ± 5.39 c	5.04 ± 0.74 c	10.16 ± 3.42 b

Note: Z1-1: herbaceous peonies grown in the 1-year plantation for one year; Z5-5: herbaceous peonies grown continuously in the 5-year plantation for five years; H5-1: herbaceous peonies replanted in the 5-year plantation for one year; Z8-8: herbaceous peonies grown continuously in the 8-year plantation for eight years; H8-1: herbaceous peonies replanted in the 8-year plantation for one year; H8-5: herbaceous peonies replanted in the 8-year plantation for five years. Data are presented as the mean ± SD. Different lowercase letters indicate significant differences at $p < 0.05$.

3.2. Changes in Root Activity

Root activity for Z5-5 and Z8-8 was significantly lower than that of Z1-1 by 50.31% and 44.53%, respectively (Figure 1). Root activity for H5-1 and H8-1 was significantly lower than that of Z1-1 by 58.04% and 56.90%, respectively. Root activity significantly increased after replanting for five years. Root activity of H8-5 significantly increased by 146.09% compared to Z5-5. H8-5 root activity significantly increased by 183.77% compared to H8-1.

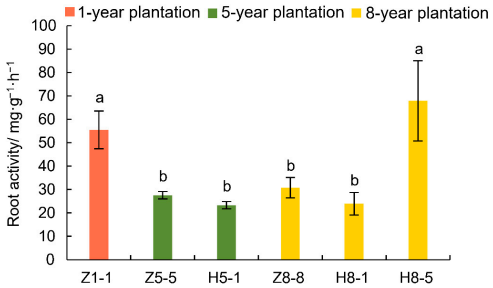

Figure 1. Root activity of herbaceous peony in different growing and replanting years. Note: Sample nomenclature is the same as Table 1. Different lowercase letters indicate significant differences at $p < 0.05$.

3.3. Changes in Soil Physical and Chemical Properties

As shown in Table 2, the pH and OM content of Z5-5 was significantly higher than those of Z1-1 and Z8-8. The EC, AP, and AK contents of Z5-5 were lower than those of Z1-1 and Z8-8. AN, AP, and AK contents in Z8-8 were significantly higher than those in Z1-1 and Z5-5. Within the same plantation, each index increased or decreased, although not all indices had statistically significant changes. The pH, AN, AP, AK, and OM contents in continuously growing herbaceous peony soil were higher than those in bulk soil. However, the EC was lower than that in bulk soil. The pH, AN, AP, AK, and OM contents in the replanted herbaceous peony soil were lower than those in the bulk soil, and the EC was significantly higher than that of the bulk soil. The pH, AN, AP, AK, and OM contents of H8-5 were lower than those of H8-1 by 1.20%, 13.15%, 14.28%, 2.15%, and 8.09%, respectively, and the EC increased significantly by 58.81%.

Table 2. Physical and chemical properties of cultivated soil in different planting and replanting years.

Sample		pH	EC (μm·cm^{-1})	AN (mg·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})	OM (g·kg^{-1})
1-year plantation	CK1	7.55 ± 0.04 b	119.27 ± 1.29 d	58.33 ± 2.02 e	59.76 ± 1.55 d	112.70 ± 11.60 bc	11.46 ± 2.96 c
	Z1-1	7.58 ± 0.04 b	77.70 ± 0.36 f	86.10 ± 1.85 c	108.88 ± 1.25 b	121.12 ± 2.08 b	19.40 ± 3.58 b
5-year plantation	CK5	7.68 ± 0.09 a	65.53 ± 0.42 g	79.57 ± 17.60 c	29.62 ± 1.65 f	103.07 ± 5.51 de	18.44 ± 2.20 b
	Z5-5	7.73 ± 0.04 a	66.17 ± 0.25 g	98.70 ± 2.52 b	53.73 ± 0.66 d	113.90 ± 5.51 bc	30.36 ± 3.93 a
	H5-1	7.54 ± 0.02 b	120.73 ± 0.93 d	73.27 ± 2.46 cd	29.14 ± 4.63 f	100.66 ± 3.61 e	17.95 ± 1.33 b
8-year plantation	CK8	7.50 ± 0.02 b	136.4 ± 1.92 c	108.50 ± 7.00 b	86.07 ± 2.29 c	122.32 ± 3.61 b	15.91 ± 4.82 bc
	Z8-8	7.57 ± 0.08 b	89.50 ± 0.36 e	123.43 ± 4.66 a	124.77 ± 5.94 a	147.59 ± 6.25 a	19.67 ± 1.31 b
	H8-1	7.49 ± 0.04 bc	150.70 ± 0.30 b	76.30 ± 5.60 cd	39.35 ± 8.13 e	111.49 ± 3.61 bcd	15.32 ± 1.47 bc
	H8-5	7.40 ± 0.07 c	239.33 ± 0.58 a	66.27 ± 4.28 de	33.73 ± 1.94 ef	109.09 ± 5.51 de	14.08 ± 2.75 bc

Note: CK1: bulk soil in the 1-year plantation; Z1-1: soil grown with herbaceous peonies for one year in the 1-year plantation; CK5: bulk soil in the 5-year plantation; Z5-5: soil grown with herbaceous peonies for five years in the 5-year plantation; H5-1: soil replanted with herbaceous peonies for one year in the 5-year plantation; CK8: bulk soil in the 8-year plantation; Z8-8: soil grown with herbaceous peonies for eight years in the 8-year plantation; H8-1: soil replanted with herbaceous peonies for one year in the 8-year plantation; H8-5: soil replanted with herbaceous peonies for five years in the 8-year plantation. pH: pH value; EC: electrical conductance; AN: alkali-hydrolyzable nitrogen; AP: available phosphorus; AK: available potassium; OM: organic matter. Data are presented as the mean ± SD. Different lowercase letters indicate significant differences at $p < 0.05$.

3.4. Changes in Soil Enzyme Activity

The Ure, Pho, and Cat activities of Z5-5 were higher than those of Z1-1 and Z8-8 (Figure 2). Within the same plantation, Inv, Ure, Pho, and Cat activities in soil from the herbaceous peony with continuous growth were higher than those of the bulk soil. There were no significant differences in the activities of the four enzymes between soil with replanted herbaceous peony and the bulk soil. The four soil enzyme activities decreased five years after replanting. Inv, Ure, Pho, and Cat activities in H8-5 decreased compared to H8-1 by 4.25%, 14.21%, 22.44%, and 15.16%, respectively.

3.5. Changes in the Soil Microbial Community

3.5.1. Changes in the Quantity of OTUs in the Soil Microbial Community

A total of 576,088 classifiable fungal and 421,097 classifiable bacterial sequences were obtained from all soil samples after high-throughput sequencing, with a mean number of 51,242 and 25,742 classifiable sequences per sample, respectively. With an increase in sequencing data, the sample rarefaction curve tended to become smooth, indicating that the sequencing depth covered most microbial communities, and the sequencing quantity was suitable (Figure S2).

OTUs were obtained for 97% sequence similarity. There were 1730 fungal OTUs in total, of which 66 OTUs were shared by all the samples, whereas 58, 49, 101, 78, 82, 73, 80, 100, and 76 OTUs were present in CK1, Z1-1, CK5, Z5-5, H5-1, CK8, Z8-8, H8-1, and H8-5, respectively (Figure 3A). There were 4423 bacterial OTUs in total, of which 978 OTUs were shared across all the samples, whereas 58, 69, 21, 24, 24, 31, 26, 40, and 17 OTUs were present in CK1, Z1-1, CK5, Z5-5, H5-1, CK8, Z8-8, H8-1, and H8-5, respectively (Figure 3B).

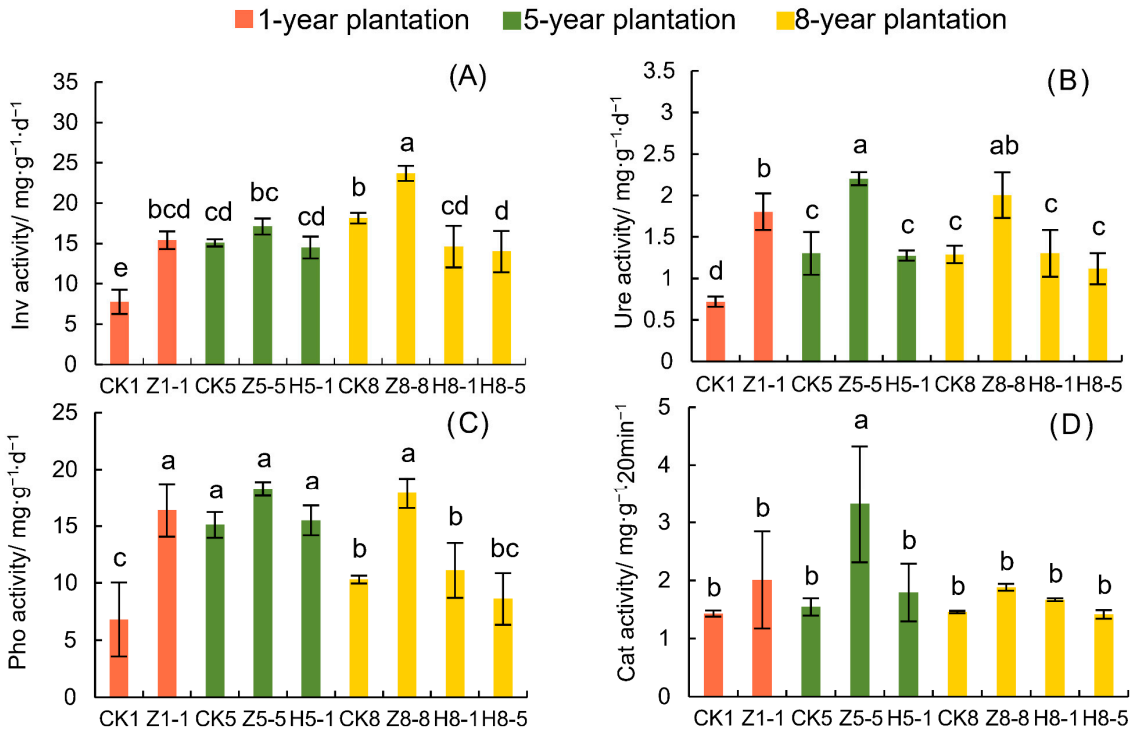

Figure 2. Soil enzyme activity under different years of growing and replanting. Note: Sample nomenclature is the same as that of Table 2. (**A**): Inv activities; (**B**): Ure activities; (**C**): Pho activities; (**D**): Cat activities. Different lowercase letters indicate a significant difference at $p < 0.05$.

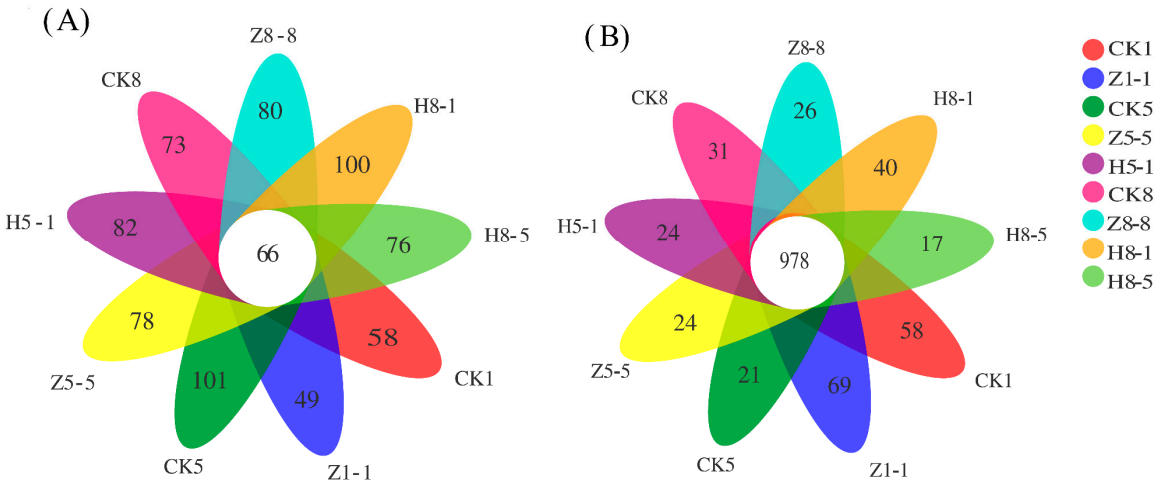

Figure 3. Quantity of OUTs in the soil microbial community in different years of growing and replanting. Note: The nomenclature for each sample is the same as that in Table 2. (**A**) Changes in OTUs quantity of fungi; (**B**) Changes in OTUs quantity of bacteria.

3.5.2. Changes in the α-Diversity Indices of Soil Microbial Communities

In this study, the α-diversity analysis of the samples showed that the coverage of all samples was higher than 0.97 (Table 3), which can better reflect the real sample situation. Among the three soils with continuously growing herbaceous peonies, the Shannon, Ace, and Chao1 indices for fungi in Z5-5 were significantly higher than those in Z1-1 and Z8-8. This indicates that the number of years of growth significantly affected the richness and diversity of fungal communities, and the extent of their effects varied widely among years of growth. The Shannon, Ace, and Chao1 indices for bacteria in the three soils

with continuous growth were Z1-1 > Z5-5 > Z8-8, indicating that continuous growth of herbaceous peonies decreased the diversity and richness of bacterial communities.

Table 3. Indices for the microbial diversity of soil planted with herbaceous peony.

Sample		Fungi				Bacteria			
		Shannon	Ace	Chao1	Coverage	Shannon	Ace	Chao1	Coverage
1-year plantation	CK1	3.55 ± 0.05 c	399.35 ± 0.87 g	413.06 ± 2.98 g	0.99 ± 0.00 a	6.82 ± 0.04 ab	3253.04 ± 0.03 h	3192.15 ± 0.14 h	0.97 ± 0.00 a
	Z1-1	2.59 ± 0.40 e	479.73 ± 0.29 f	495.02 ± 2.85 f	0.99 ± 0.00 a	6.92 ± 0.04 a	3522.64 ± 0.34 a	3481.84 ± 0.06 b	0.97 ± 0.00 a
	CK5	4.63 ± 0.08 a	646.47 ± 1.76 b	651.02 ± 0.02 b	0.99 ± 0.00 a	6.63 ± 0.2 c	3295.89 ± 2.09 f	3259.98 ± 0.64 g	0.97 ± 0.00 a
1-year plantation	Z5-5	4.46 ± 0.11 a	646.44 ± 0.06 b	650.37 ± 1.15 b	0.99 ± 0.00 a	6.73 ± 0.06 bc	3348.13 ± 0.14 e	3337.28 ± 0.19 d	0.97 ± 0.00 a
	H5-1	4.51 ± 0.00 a	608.21 ± 4.96 d	619.33 ± 1.00 c	0.99 ± 0.00 a	6.76 ± 0.14 abc	3449.20 ± 0.09 c	3491.00 ± 0.01 a	0.97 ± 0.00 a
	CK8	4.54 ± 0.06 a	586.58 ± 0.35 e	594.03 ± 0.13 e	0.99 ± 0.00 a	6.81 ± 0.03 abc	3456.24 ± 0.03 b	3433.39 ± 0.00 c	0.97 ± 0.00 a
1-year plantation	Z8-8	3.12 ± 0.02 d	618.15 ± 0.16 c	616.26 ± 0.75 d	0.99 ± 0.00 a	6.64 ± 0.01 bc	3290.89 ± 0.00 g	3279.62 ± 0.08 f	0.97 ± 0.00 a
	H8-1	4.38 ± 0.01 a	677.35 ± 0.04 a	699.86 ± 0.87 a	0.99 ± 0.00 a	6.79 ± 0.12 abc	3372.85 ± 0.09 d	3304.96 ± 0.06 e	0.97 ± 0.00 a
	H8-5	3.83 ± 0.01 b	588.53 ± 0.09 e	593.72 ± 0.06 e	0.99 ± 0.00 a	6.68 ± 0.03 bc	3106.45 ± 0.00 i	3082.50 ± 0.17 i	0.97 ± 0.00 a

Note: The nomenclature for each sample is the same as that in Table 2. Data in the table are means ± SD. Different lowercase letters in the same column indicate a significant difference at $p < 0.05$.

Within the same plantation, compared with bulk soil, the Shannon index for Z1-1 and Z8-8 fungi decreased significantly, whereas the Ace and Chao1 indices significantly increased, and the Shannon, Ace, and Chao1 indices for Z5-5 fungi did not change significantly. Compared with bulk soil, the bacterial Ace, and Chao1 indices for Z1-1 and Z5-5 increased significantly, and the bacterial Shannon index increased by 1.47% and 1.51%, respectively. While Ace, and Chao1 indices for Z8-8 decreased significantly, and the bacterial Shannon index decreased by 2.50%. The Shannon, Ace, and Chao1 indices for H8-5 fungi and bacteria were lower than those of CK8 and H8-1.

3.5.3. Changes in Soil Fungal Community Composition

The fungal isolates in the test samples comprised of 16 phyla, 44 classes, 94 orders, 221 families, 438 genera, and 705 species. At the phylum level (Figure 4), except for the unclassified _k_fungus, the fungi with an average abundance greater than 1% included *Ascomycota* (70.92%), Basidiomycota (15.01%), *Mortierellomycota* (8.37%), and *Chytridiomycota* (8.37%). Among the three soils with continuously growing herbaceous peonies, the relative abundance of *Ascomycota* of Z8-8 was the lowest and that of *Basidiomycota* was the highest. The relative abundance of *Ascomycota* of Z1-1 was the highest and that of *Basidiomycota* was the lowest. The relative abundance of *Ascomycota* of H8-5 decreased by 26.76% compared with H8-1, and that of *Basidiomycota* increased by 431.71% compared with H8-1.

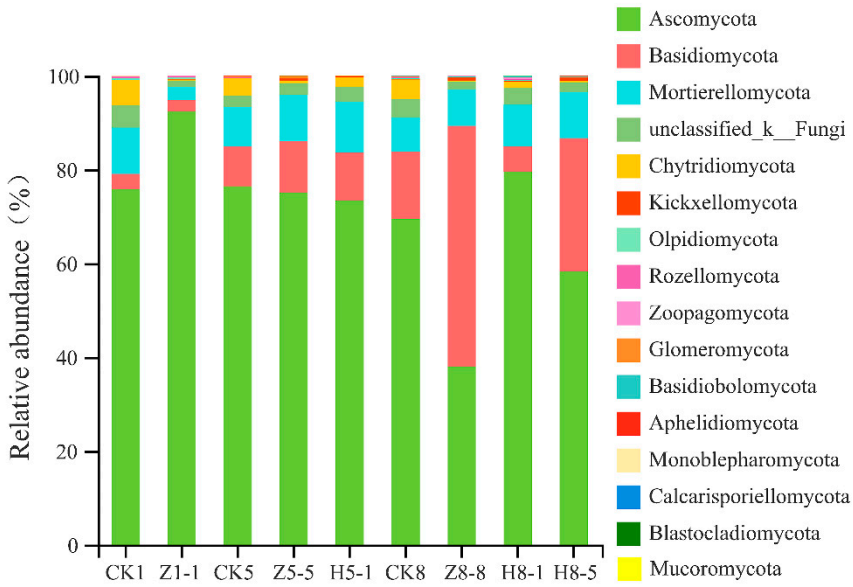

Figure 4. Community composition of fungi at the phylum level in soil in different years of growing and replanting. Note: The nomenclature for each sample is the same as that in Table 2.

Figure 5 showed that in addition to genera with unknown classification, those with an average abundance greater than 1% included *Kernia* (10.92%), *Apiotrichum* (8.69%), *Mortierella* (8.03%), *Talaromyces* (3.63%), *Gibberella* (3.33%), and *Hymenula* (3.33%), for a total of 18 genera. *Kernia* was mainly found in the 1-year plantation. Compared with CK1, the relative abundance of *Kernia* of Z1-1 increased by 98.77%. The relative abundance of *Apiotrichum* was low in the bulk soils and gradually increased after herbaceous peony cultivation. Compared with H8-1, the relative abundance of *Gibberella* of H8-5 increased by 145.09%, and that of *Chaetomium* of H8-5 decreased by 30.54%. *Barnettozyma* and *Schizothecium* were predominantly present in Z5-5.

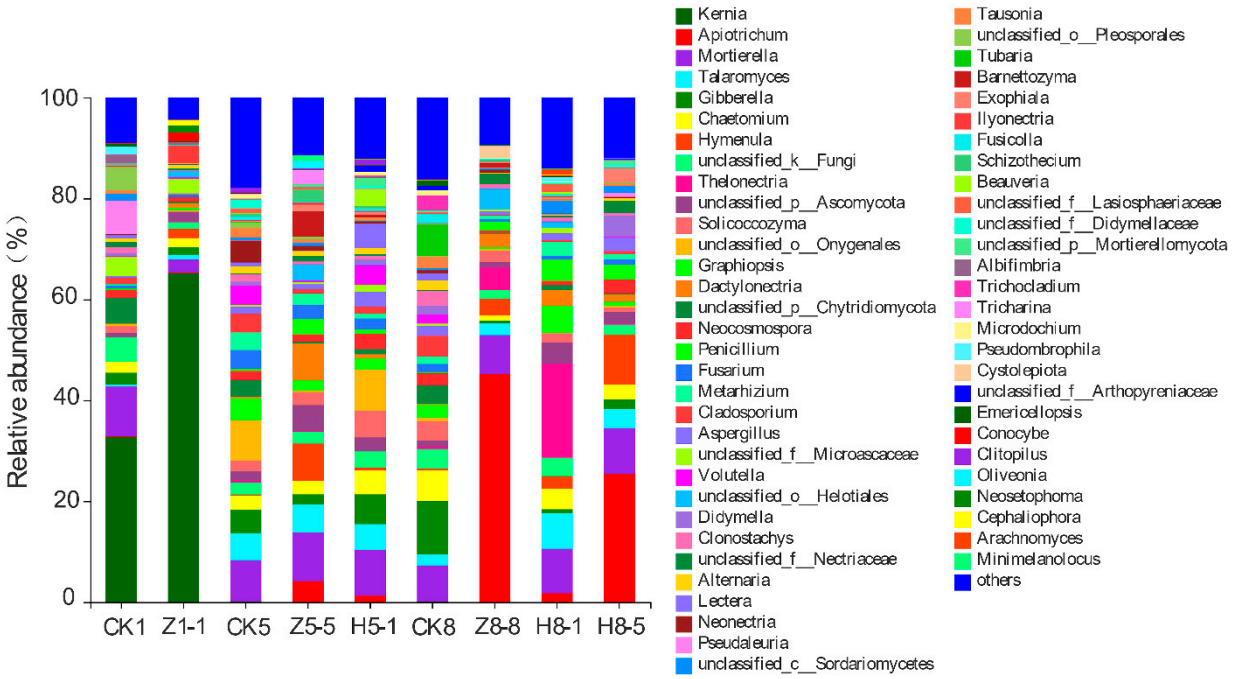

Figure 5. Community composition of fungi at the genus level in soil in different years of growing and replanting. Note: The nomenclature of each sample is the same as that in Table 2.

In addition to species with unknown classification (Figure 6), species with an average abundance greater than 1% included *Mortierella alpina* (4.39%), *Kernia geniculotricha* (3.48%), *Gibberella intricans* (2.99%), *Hymenula cerealis* (2.90%), and *Thelonectria rubrococca* (2.44%), for a total of six species. Compared with H8-1, the relative abundance of *Hymenula cerealis* and *Didymella heteroderae* of H8-5 increased by 290.25% and 1380.76%, respectively, and that of *Thelonectria rubrococca* of H8-5 decreased from 18.54% to 0%. The relative abundance of *Barnettozyma californica* in Z5-5 was the highest at 5.04%. *Clitopilus* sp., *Psathyrella kellermanii*, and *Cystobasidium minuta* were only present in H8-1 and H8-5 (Table S1).

3.5.4. Changes in Soil Bacterial Community Composition

A total of 40 phyla, 122 classes, 284 orders, 440 families, 793 genera, and 1626 species of bacteria were identified in the test samples. At the phylum level (Figure 7), the average abundance of the test samples was greater than 1% for *Actinobacteriota* (24.87%), *Proteobacteria* (21.96%), *Acidobacteriota* (17.47%), *Chloroflexi* (12.13%), *Firmicutes* (5.28%), *Bacteroidota* (3.40%), and others, for a total of nine phyla. Compared with H8-1, the relative abundance of *Acidobacteria* of H8-5 increased by 31.05%, and that of *Proteobacteria* and *Firmicutes* of H8-5 decreased by 3.91% and 39.33%, respectively.

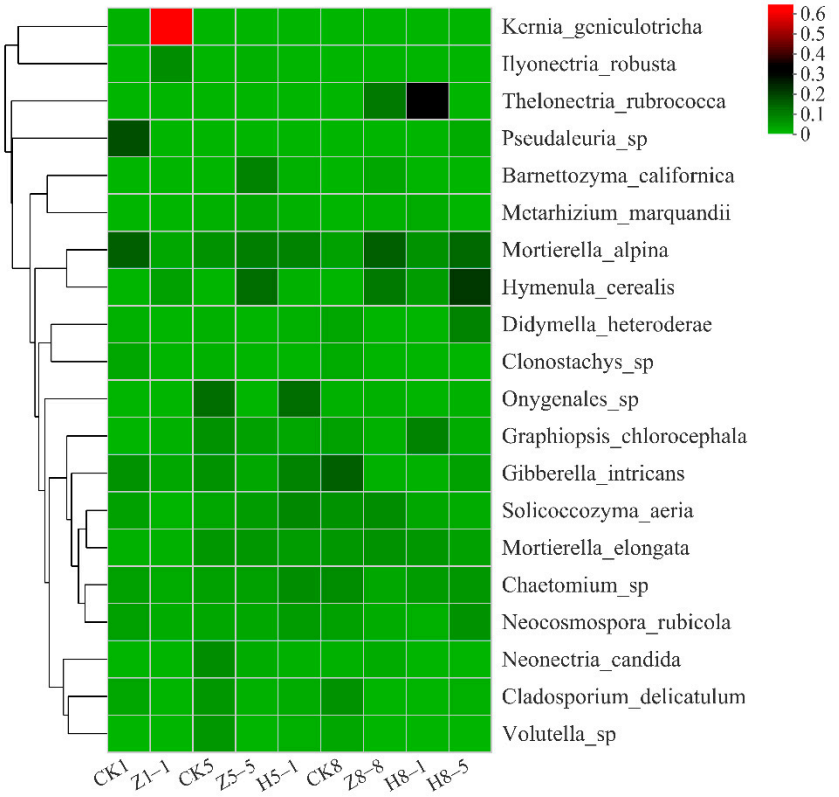

Figure 6. Heat map of fungi species with top 20 relative abundances in the soil in different years of growing and replanting. Note: The nomenclature for each sample is the same as that in Table 2.

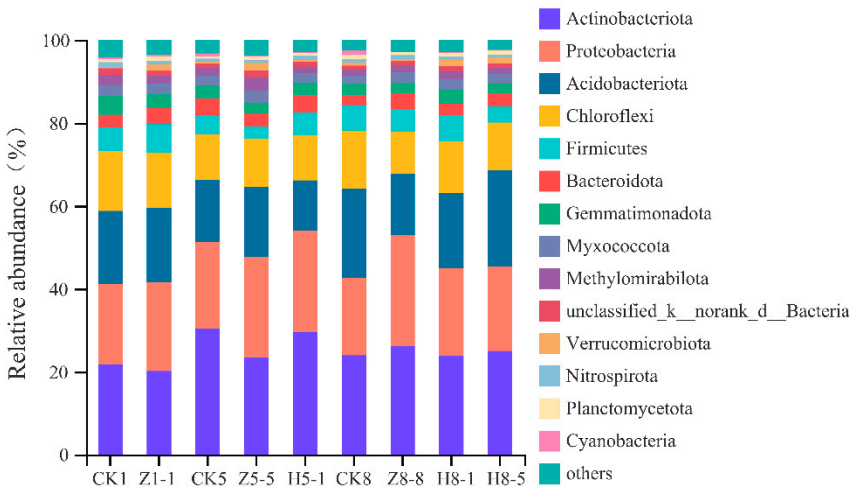

Figure 7. Community composition of bacteria at the phylum level in soil in different years of growing and replanting. Note: The nomenclature of each sample is the same as that in Table 2.

At the genus level, 57.54% of the bacterial genera were unknown (Figure 8). In addition to the unknown genera, those with an average abundance greater than 1% for the test samples included *Bacillus* (2.66%), *Arthrobacter* (2.41%), *Gaiella* (2.18%), *RB41* (1.99%), *Sphingomonas* (1.63%), *MND1* (1.29%), *Nocardioides* (1.15%), and *Pseudomonas* (1.00%), for a total of eight genera. The relative abundance of *Pseudomonas*, *Pseudolabrys*, and *Flavobacterium* was higher in the three soils with continuously growing herbaceous peonies. Compared with H8-1, the relative abundances of *Bacillus*, *Arthrobacter*, *Sphingomonas*, *Pseudomonas*, and *Pseudolabrys* in H8-5 decreased by 28.36%, 5.71%, 22.63%, 7.32% and 42.86%, respectively.

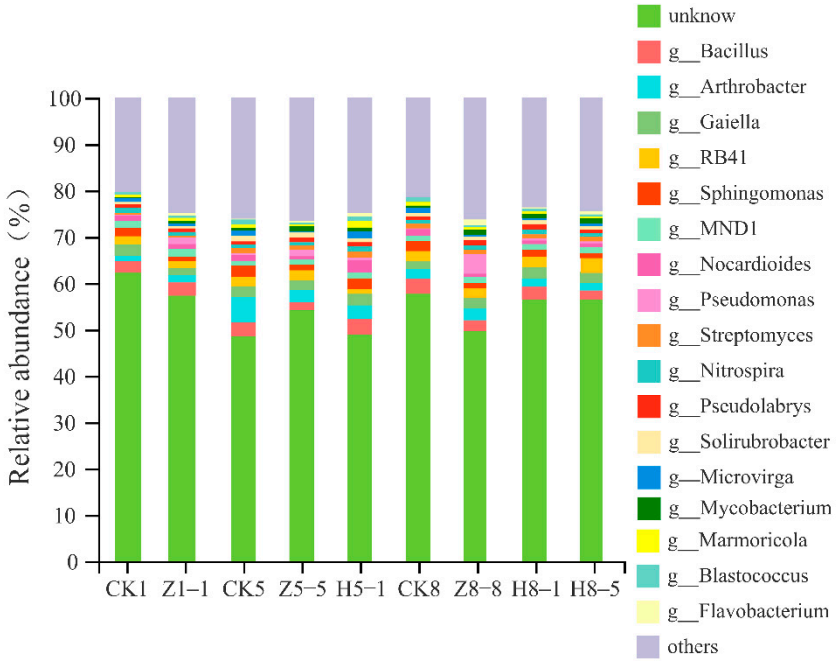

Figure 8. Community composition of bacteria at the genus level in soil in different years of growing and replanting. Note: The nomenclature of each sample is the same as that in Table 2.

At the species level, 90.87% of all samples were unknown. Except for the unknown species, the top 20 species with relative abundances from the test samples were shown in Figure 9.

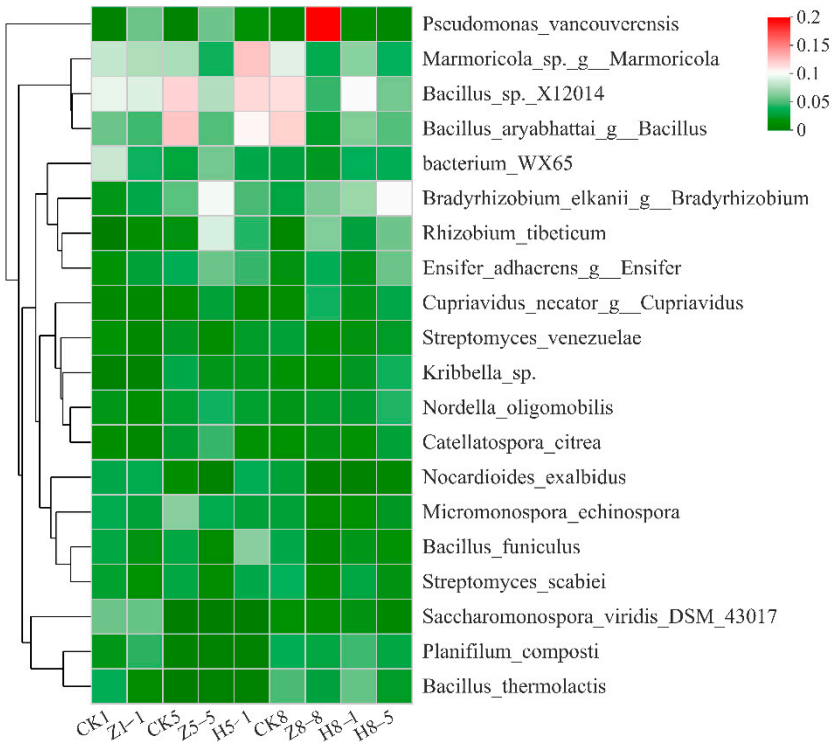

Figure 9. Heat map of bacterial species with top 20 relative abundances in soil in different years of growing and replanting. Note: The nomenclature for each sample is the same as that in Table 2.

The relative abundances of *Pseudomonas vancouverensis* and *Rhizobium tibeticum* were higher in the soils with continuously growing herbaceous peonies than those in the bulk

and replanted soils. Compared with H8-1, the relative abundances of *Marmoricola* sp. and *Bacillus* sp. X12014 of H8-5 decreased by 39.47% and 32.65%, respectively. *Azorhizobium doebereinerae*, *Legionella feeleii*, *Verrucomicrobiaceae bacterium*, and *Sphaerobacter thermophiles* DSM_20745 were only present in H8-5 (Table S2).

3.5.5. Correlation between Soil Microbial Community and Soil Environmental Factors

Redundancy analysis (RDA) revealed a correlation between soil environmental factors and microorganisms. The first two axes of the RDA explained 82.28% and 60.45% of the structural differences in soil fungal community composition in Figure 10A,B, respectively. In addition, the first two axes of the RDA explained 56.86% and 53.12% of the structural differences in soil bacterial community composition in Figure 10C,D, respectively. The RDA plot showed a clear separation in the space among the samples. Fungal communities from H8-5 were separated from those from H5-1 and H8-1 along the second RDA axis. This separation was predominantly influenced by AK, AN, pH, Ure, Pho, and Inv. The structure of H8-5 communities was related to higher AK, AN, Ure, Pho, Inv, and lower pH values. Bacterial communities from H5-1, H8-1, and H8-5 were separated along the first RDA axis, and this separation was mainly influenced by EC, pH, and Pho. The structure of the H8-5 communities was related to higher EC and lower pH values.

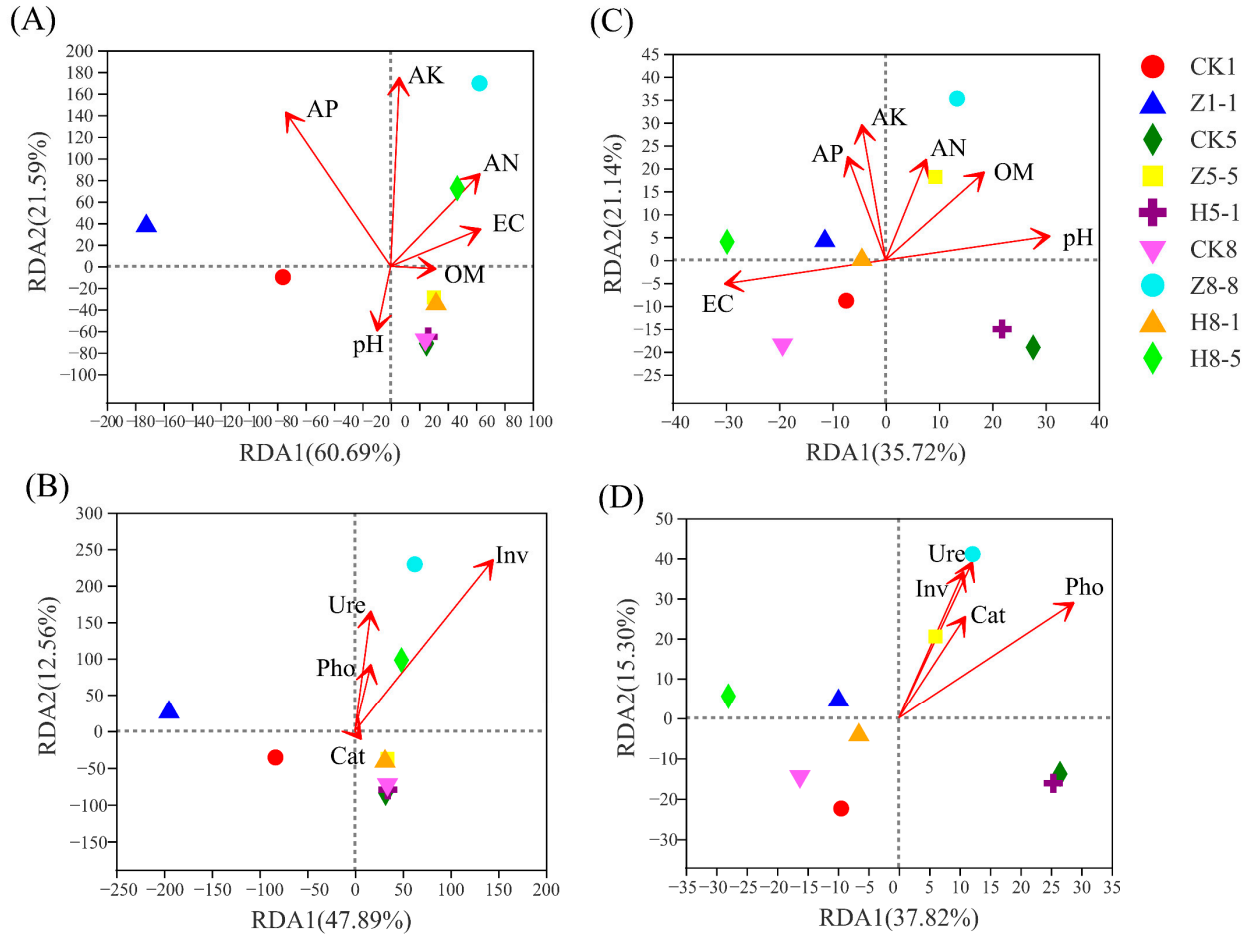

Figure 10. Redundancy analysis (RDA) of microbial community changes with soil parameters. Note: (**A**): RDA of fungi community changes with physical and chemical properties of soil; (**B**): RDA of fungi community changes with soil enzyme activity. (**C**): RDA of bacteria community changes with physical and chemical properties of soil; (**D**): RDA of bacteria community changes with soil enzyme activity. The nomenclature for each sample is the same as that in Table 2. pH: pH value; EC: electrical conductance; AN: alkali-hydrolyzable nitrogen; AP: available phosphorus; AK: available potassium; OM: organic matter; Inv: invertase; Ure: urease; Pho: phosphatase; Cat: catalase.

4. Discussion

4.1. Herbaceous Peony Growth and Development

Adversity stress is a general term for various environmental factors that are unfavorable to plant growth and development [29]. The replanting problem of herbaceous peony is actually a kind of adversity stress. Adversity stress affects the growth and development plants. Plants respond to stress by changing their morphology [30]. Ma and Peng [31] found that replanted tree peonies showed slow growth, poor stress resistance, produced fewer flowers, and early senescence. Sun [32] showed that growth indices such as plant height and stem diameter decreased with an increase in years after replanting. The present study showed strong growth and development in herbaceous peonies within eight years. The growth and development of herbaceous peonies were limited by replanting, and they almost died after being replanted for five years.

Root activity is an index expressing root-physiological metabolism, reflecting the ability of the root system to absorb soil nutrients [33]. Continuous cropping significantly reduced root activity in watermelon and potatoes [34,35]. The results showed that the root activity of herbaceous peonies one year after replanting was significantly lower than that of one year of growth. However, root activity five years after replanting was significantly higher than that after five years of continuous growth. Root activity was also significantly higher than plants one year after replanting possibly because the soil environment deteriorated after replanting. After short-term replanting, the plants failed to adapt to the environment and root activity decreased. After long-term replanting to adapt to the environment to increase the absorptive capacity of plants, the root activity increased.

4.2. Effects of Different Growing and Replanting Years on Soil Environment

The soil environment plays a crucial role in plant growth and development. Continuous growing or replanting changes the physical and chemical properties of the soil and influences enzyme activity [36–38]. Physicochemical properties such as soil pH and EC are important indicators of soil health [39]. A decrease in soil pH represents an increase in the concentration of H^+ in the soil, reducing soil biological activity and affecting plant growth [40]. A higher soil EC denotes a higher soil salt content, and a lower EC improves plant growth and vitality [41]. Soil OM contributes to the formation of soil aggregates, improves soil physical and chemical properties, and promotes the absorption of nutrients [42]. Soil AN, AP, and AK can be directly absorbed and used by plants, and their contents play a key role in plant growth [43]. Soil enzyme activity reflects the soil material level, energy metabolism, and soil quality [44]. In our study, soil pH and AN, AP, AK, OM, Inv, Ure, Pho, and Cat contents in soil with continuous herbaceous peony growth were higher than those in bulk soil, whereas the EC was lower, indicating the presence of sufficient soil nutrients, strong metabolism and nutrient transformation, sufficient nutrient supply, and positive health conditions within eight years of continuous peony growth. Meanwhile, pH and AN, AP, AK, and OM contents in replanted soil were lower than those in bulk soil, and the EC was higher than that in bulk soil, showing that the soil environment deteriorated and nutrient content decreased after herbaceous peony replanting. This may be the reason for the herbaceous peony replanting problem. Soil EC, AP, and AK content were the lowest, with continuous peony growth for five consecutive years, indicating that peonies have a high demand for phosphorus and potassium, and timely fertilization should be considered during production.

4.3. Effects of Different Growing and Replanting Years on Soil Microbial Adiversity

The Shannon index reflects the diversity of microbial communities, and the Chao1 and Ace indices present the species richness of microbial communities in soils. The larger the Shannon, Chao1, and Ace indices, the higher the community diversity and richness [45]. Microbial community diversity and richness are important indicators that reflect soil quality and plant health. Their enhancement is beneficial for increasing functional redundancy within the microbiome and protecting plants from soil-borne diseases [46,47].

In our study, fungal community diversity in herbaceous peony soils with one and eight years of continuous growth was lower, and the richness was higher than in bulk soils. This may be because of the alteration of the rhizospheric soil environment during herbaceous peony growth, which enriched the specific fungal community in the rhizosphere and inhibited the growth of other soil fungi [48]. Fungi diversity and richness in the soil of herbaceous peonies with five years of continuous growth were not significantly different from those of bulk soil. This is likely because the soil community structure was stable, allowing vigorous growth in herbaceous peonies over five years of continuous growth.

Bacterial diversity and richness in soils with one and five years of continuous herbaceous peony growth were higher than those in bulk soils. Bacterial diversity and richness were lower in soils with eight years of continuous herbaceous peony growth than those in bulk soils. This may be due to the exuberant root growth of herbaceous peonies in the early stage that releases a large amount of root exudates into the soil. Owing to the effects of nutrient enrichment, the nutrient content in the rhizosphere is high, which promotes bacterial growth and development [49]. The allelopathic chemicals secreted by herbaceous peony root systems showed long-term accumulation, which destroyed the soil environment and inhibited bacterial growth. The decrease in bacterial diversity and richness may weaken herbaceous peonies for eight consecutive years.

Microbial community diversity and richness of the soil after replanting herbaceous peonies for five years significantly decreased. Soil microbial community diversity and richness in the replanted soil from ginseng, *Atractylodes macrocephala* and apple also decreased [50–52], consistent with the findings of our study.

Changes in soil physicochemical properties and enzyme activities can lead to changes in microbial community diversity [53]. Bell, et al. [54] found that available nitrogen drives soil bacteria to gather during plant growth. Li, et al. [55] found that microbial community diversity and richness in farmland with long-term application of organic fertilizer, nitrogen, phosphorus, and potassium were significantly higher than those without fertilization. When herbaceous peonies were replanted over longer timescales, soil nutrient content and enzyme activity decreased, soil quality decreased, and microbial diversity and richness decreased, affecting the microbial community's stability and inhibiting aboveground plant growth.

4.4. Effects of Different Growing and Replanting Years on Soil Fungal Community Composition

Ascomycota and *Basidiomycota* play a dominant role in soil fungal communities, *Ascomycota* predominantly decomposes organic matter, whereas *Basidiomycota* decomposes lignin and cellulose and promotes soil nutrient cycling [56]. In the present study, *Kernia* was only present in the 1-year plantation, and was a saprophytic fungus isolated from animal dung [57]. The application of a large amount of organic fertilizer before herbaceous peony planting may have caused its presence in the 1-year plantation. *Kernia* can accelerate the decomposition of nutrients in the fertilizer. Its ecological functions have not yet been reported and require further research. In addition, the relative abundance of *Apiotrichum* increased after herbaceous peony cultivation. It is likely that *Apiotrichum* plays an essential role in peony growth and its ecological functions need to be investigated further.

Pathogenic fungi such as *Fusarium* increased significantly after the replanting of *Rehmannia* and apple [15,58]. We found that the relative abundance of *Gibberella* increased and the relative abundance of *Chaetomium* decreased after long-term replanting. *Gibberella* can infect many food and commercial crops, causing plant diseases such as wheat scab [59]. *Chaetomium* can protect the plant body by inhibiting plant pathogens through mycoparasitism and producing antibiotics such as chaetomin. It also promotes the absorption of nitrogen and phosphorus by plants from the soil [60–62]. The increase in the relative abundance of soil pathogenic fungi and the decrease in beneficial fungi may have caused a decrease in the quality and yield of herbaceous peony replanting. We found that the relative abundance of the beneficial fungi *Barnettozyma* and *Schizothecium* was higher in soils with herbaceous peony growing continuously for five years than in other soils. *Barnettozyma*

is a biocontrol fungus with an antagonistic effect on *Verticillium* [63]. *Schizothecium* can promote plant growth, improve their tolerance to environmental stress, and can inhibit banana wilt [64]. Therefore, *Barnettozyma* and *Schizothecium* likely play important roles in promoting herbaceous peony growth.

The relative abundance of the pathogenic fungus *Hymenula cerealis* [65] increased in the long-term replanting soil, possibly the key species causing the herbaceous peony replanting problem. Soil with continuous peony growth for five years had the highest relative abundance of the beneficial fungus *Barnettozyma californica* [63], potentially contributing to herbaceous peony growth. The relative abundance of *Thelonectria rubrococca* and *Didymella heteroderae* changed in the replanted soil. *Clitopilus* sp., *Psathyrella kellermanii*, and *Cystobasidium minuta* were only found in the replanted soil, suggesting that they may be associated with the replanting problems of herbaceous peonies.

4.5. Effects of Different Growing and Replanting Years on Soil Bacterial Community Composition

In this study, the dominant bacterial phyla in the test soil were *Actinobacteriota*, *Proteobacteria*, *Acidobacteriota*, *Chloroflexi*, and *Firmicutes*, which were the most common phyla in the bacterial community. *Proteobacteria* is a gram-negative bacterium that propagates rapidly and predominantly occurs in a nutrient-rich environment [56]. *Acidobacteria* are oligotrophic bacteria that are usually abundant in poor soils [66]. *Firmicutes* are positively correlated with crop health [67]. The relative abundance of *Proteobacteria* and *Firmicutes* decreased, and that of *Acidobacteria* increased during long-term replanting. It was speculated that the soil environment of herbaceous peony replanting deteriorated, and the nutrient content decreased, increasing oligotrophic bacteria and decreasing eutrophic bacteria, and changing the microbial community structure.

The relative abundance of *Pseudomonas*, *Pseudolabrys*, and *Flavobacterium* in soil with continuously growing herbaceous peonies was higher than that in other soils. In contrast, the relative abundance of *Bacillus*, *Arthrobacter*, *Sphingomonas*, *Pseudomonas*, and *Pseudolabrys* was lower in long-term replanting soils. *Bacillus* and *Pseudomonas* inhibit pathogens and promote plant growth [68,69]. *Arthrobacter* degrades various chemical pollutants in the soil, especially those that are difficult to decompose [69]. *Sphingomonas* is predominantly involved in degrading aromatic compounds and toxic substances in the carbon cycle, which can improve the soil environment [70]. *Pseudolabrys* is ubiquitous in hydrocarbon-rich soils and are hydrocarbon-degrading bacteria [66]. *Flavobacterium* has a growth-promoting effect on plants, and some groups can induce a higher activity of chitinase to inhibit the growth of phytopathogenic fungi [71]. Therefore, the relative abundance of beneficial bacteria in the replanted soil decreased, which reduced the inhibition of pathogens, increased the abundance of pathogens, and an imbalance of soil microbiology, resulting in the limited growth and development of herbaceous peonies and even died.

The relative abundance of beneficial bacteria, *Pseudomonas vancouverensis* [72] and *Rhizobium tibeticum* [73] in soil with continuously growing peonies was higher than that in bulk and replanted soil. It is likely to promote the growth of herbaceous peonies. The relative abundance of *Marmoricola* sp. and *Bacillus* sp. X12014 were significantly lower in long-term replanting soils. Their decrease is likely disadvantageous to peony growth. *Azorhizobium doebereinerae*, *Legionella feeleii*, *Verrucomicrobiaceae bacterium*, and *Sphaerobacter thermophiles* DSM_ 20745 only occurred in the replanted soil and their ecological functions need to be further studied.

5. Conclusions

The growing and replanting period for herbaceous peonies significantly affected the growth, soil environment, and soil microbial community structure. Long-term replanting of herbaceous peonies inhibited their growth, decreased the soil nutrient content and enzyme activity, and resulted in soil environmental deterioration. Long-term replanting reduced the diversity and richness of the soil microbial community, stability of the soil micro-ecosystem, and relative abundance of beneficial microorganisms. However, it increased the relative

abundance of harmful soil microorganisms. These changes led to a change in the microbial community structure and an imbalance in the soil microecology and are potentially the main reason for the herbaceous peony replanting problem. Some of the bacteria and fungi that were not described and identified in this study by high-throughput sequencing sequence analysis need to be further identified using other analytical tools. In the future, more in-depth studies on beneficial and harmful bacterial species related to the growth and development of herbaceous peonies should be conducted.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020220/s1, Figure S1: Soil sample; Figure S2: Rarefaction curves of OTUs in each soil sample; Table S1: Relative abundance of fungi communities in soil under different years of planting and replanting at the species level; Table S2: Relative abundance of bacterial communities in soil under different years of planting and replanting at the species level.

Author Contributions: Y.L. and Z.L. co-designed and undertook most of the study, analyzed the data, and wrote the paper. L.Y., X.Y., Y.S., X.L. and L.D. participated in tests and data analysis. C.Z. provided comments on the first draft of this paper. D.Z. and X.S. co-designed the study, and revised drafts of the manuscript. All authors have read and agreed to the published version of the manuscript.

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