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Response of Typical Artificial Forest Soil Microbial Community to Revegetation in the Loess Plateau, China

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Abstract

This study aims to analyze the differences in soil bacterial community structure under different vegetation restoration types, and to explore the role of microorganisms in the process of vegetation restoration on the soil ecosystem of the Grain for Green area in the Loess Plateau. High-throughput sequencing technology was used to analyze the alpha diversity of soil bacteria, community structure characteristics, and the correlation between soil environmental factors and bacterial communities in different artificial *Hippophae rhamnoides* forests. Soil microbial C and N show a decreasing trend with an increase in the 0–100 cm soil layers. The results indicated that the bacterial communities comprised 24 phyla, 55 classes, 110 orders, 206 families, 348 genera, 680 species, and 1989 OTUs. Additionally, the richness indices and diversity indices of the bacterial community in arbor shrub mixed forest are higher than those in shrub pure forest, and the indices of shrub forest on sunny slope are higher than those on shady slope. Across all samples, the dominant groups were Actinobacteria (37.27% on average), followed by Proteobacteria (23.91%), Acidobacteria (12.75%), and Chloroflexi (12.27%). Soil nutrient supply, such as TOC, TN, AN, AP, and AK, had crucial roles in shaping the composition and diversity of the bacterial communities. The findings reveal that vegetation restoration significantly affected soil bacterial community richness and diversity. Furthermore, based on the results, our data provide a starting point for establishing soil bacterial databases in the Loess Plateau, as well as for the plants associated with the vegetation restoration.

Keywords: high-throughput sequencing; soil bacterial community; soil properties factor; the Loess Plateau

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1. Introduction

Ecological restoration is a complex process that involves the improvement of green vegetation coverage, prevention of soil erosion, and reduction in natural disasters. Currently, revegetation is a hot topic of research in ecology, encompassing soil physical and chemical properties and soil and water conservation [1], ecological stoichiometric characteristics of vegetation and soil [2], soil nutrient element preservation [3], biodiversity of undergrowth vegetation [4], and soil microbial communities [5]. As the agents of decomposition in the ecosystem, soil microorganisms play a key role in forest succession and nutrient cycling.

They are the link between the aboveground and underground components of the ecosystem and represent an important index for the evaluation of ecosystem restoration [6,7]. Additionally, relevant studies have reported the species and soil physical and chemical properties of soil microbial community composition and diversity [8–10].

Revegetation is not only the first step in ecosystem reconstruction but also an important measure of ecological environment management and soil and water conservation on the Loess Plateau, and it can effectively reduce soil erosion and improve soil quality [11]. Since 1998, the government has adopted a development strategy based on "closing hills and returning farmland, afforestation to grass, indoor-sheep production, and planting trees to poverty eradication." Long-term vegetation restoration has achieved good macro effects, and vegetation coverage and soil quality have been improved to varying degrees [12]. In recent years, the revegetation project on the Loess Plateau has increased the area of vegetation considerably. The area of increased forest land and grassland on the Loess Plateau can reach up to 8954 square kilometers and 5235 square kilometers, respectively [13,14]. Additionally, an increasing number of studies have examined the revegetation of the returned farmland in this area [15]. In general, these studies focus on the impacts of revegetation on soil properties, changes in species diversity, the benefits of soil and water conservation, and the allocation mode of revegetation in the process of land restoration. However, there has been no systematic analysis of the interaction between revegetation, soils, and microorganisms in the process of land restoration.

Vegetation restoration influences soil microbial community structure by affecting soil physical and chemical properties. For instance, Zhao et al. [16] indicated that vegetation recovery can enhance soil water retention and aeration, which are beneficial for microbial growth and reproduction. Additionally, vegetation recovery reduces soil erosion and loss, protecting soil resources and providing a more stable habitat for microorganisms [17,18]. Therefore, considering the role and needs of soil microorganisms during vegetation restoration, strengthening research on soil microorganisms to improve ecological benefits of various ecosystems, increase biological productivity, and protect microbial species diversity within different ecosystems has become one of the most critical issues currently.

Therefore, to analyze the differences in soil bacterial community structure under different vegetation restoration types on the soil ecosystem of the Grain for Green Project area in the Loess Plateau, the present study investigated the dynamics of soil properties and the structure of soil bacterial communities using 16S rRNA high-throughput sequencing.

2. Materials and Methods

2.1. Study Site and Sampling

The vegetation composition is dominated by the flora of North China. Since the implementation of the Grain for Green Program in the 1990s, vegetation coverage in that area has significantly increased. *Hippophae rhamnoides*, which is a pioneer and associated species of vegetation structure in the Loess Plateau, has clear ecological and economic benefits. Therefore, this study focuses on pure stands and mixed *H. rhamnoides* forests.

Typical sample plots were selected in stands, and 20 m × 20 m arbor sample plots were set up to observe and record the position (coordinate information, altitude) and site condition (such as slope and canopy closure) of the sample site in August 2017. Table 1 shows the specific situation. At each sampling site, soil was collected from five soil cores in layers of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm. The surface soil samples were collected from each quadrat at random through a 2 mm sieve and mixed immediately to form a single soil sample. Then, it was divided into two parts. One part was placed into a 15 mL centrifuge tube, placed into a liquid N canister, transferred to the

laboratory, and stored in a freezer at −80 °C for microbial analyses. The other part was subjected to air-drying treatment and used to determine soil nutrients [19].

Table 1. Site conditions and vegetation composition.

Plot Code	Forest Types	Geographical Location	Stand Age/Year	Altitude/m	Slope Aspect	Slope/°	Main Vegetation
HrN	*H. rhamnoides* pure forest (shady slope)	108°14′16.132″ E 36°53′45.027″ N	20	1504	North	24	*Carex lanceolata Boott*, *Carduus nutans* L., *Buddleja lindleyana Fortune*
HrS	*H.rhamnoides* pure forest (Sunny slope)	108°14′16.132″ E 36°53′45.027″ N	20	1521	South	15	*Carex lanceolata Boott*, *Potentilla strigosa Pall ex Pursh ex Pursh*, *Artemisia capillaris*, *Saussurea japonica* (Thunb.) DC.
HrBo	*H.rhamnoides* and *Biota orientalis* mixed forest	108°14′16.132″ E 36°53′45.027″ N	20	1433	South	28	*Carex lanceolata Boott*, *Kalimeris indica* (Linn.) Sch., *Phragmites communis*, *Deyeuxia arundinacea* (L.) *Beauv.*, *Hedyotis auricularia* L.
HrPt	*H.rhamnoides* and *Pinus tabulaeformis* mixed forest	108°14′16.132″ E 36°53′45.027″ N	20	1408	South	18	*Carex lanceolata Boott*, *Potentilla strigosa Pall ex Pursh ex Pursh*, *Artemisia sacrorum Ledeb.*, *Lespedeza bicolor* Turcz.
GL	Grassland	108°14′16.132″ E 36°53′45.027″ N	—	1408	South	18	*Kalimeris indica* (Linn.) Sch., *Potentilla strigosa Pall ex Pursh ex Pursh*, *Lespedeza bicolor Turcz.*, *Pterocypsela indica* (L.) Shih, *Artemisia annua*

2.2. Soil Sample Determination

2.2.1. Sequencing of Soil Microbial Genes

Extraction of DNA: Following the manufacturer's instructions, the genomic DNA was isolated from 0.5 g of each soil sample from each sample site using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Union City, CA, USA). Extracts of three technical repeats were mixed into a single DNA sample. Extracted genomic DNA was detected by 1% agarose gel electrophoresis. DNA concentration and purity were measured with an ultramicro spectrophotometer (Nano Drop2000, Thermo Fisher, Waltham, MA, USA).

PCR amplification: Based on previous reports, the primers 338F-806R for the 16S rRNA of bacteria were used. Amplified products were detected by 2% agarose gel electrophoresis, recovered from the gel using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), washed with Tris-HCl, and verified by 2% agarose gel electrophoresis.

PCR products were quantified by the QuantiFluorTM-ST Fluorometer (Promega Biotech, Beijing, China) and the samples adjusted as required for sequencing. High-throughput sequencing was performed using the Illumina Miseq PE300 platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

2.2.2. Soil Microbial Carbon and Nitrogen Determination

The soil microbial biomass of C and N was determined using fumigation extraction [13]. Briefly, 25 g of the oven-dry equivalent of field-moist soil was fumigated at 25.8 °C for 24 h with $CHCl_3$. The soil was added to 100 mL of 0.5 M potassium sulfate by shaking at 200 rpm for 1 h and then filtered after fumigant removal. Another 25 g of non-fumigated soil was simultaneously extracted.

2.2.3. Determination of Soil Physical and Chemical Properties

The contents of soil organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and total kalium (TK) were determined using the vario EL cube CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil alkali-hydro N (AN) was measured using the alkali-hydrolyzed diffusion method, and available phosphorus (AP) was determined by a UV–Visible Spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). Available kalium was determined by an iCAP 6300 ICP-OES Spectrometer (Thermo Fisher, Waltham, MA, USA).

2.3. Data Processing and Analysis

2.3.1. Basic Data Processing

All data were analyzed using IBM SPSS Statistics Version 27, R version 4.0.3, and the free online platform of Majorbio Cloud Platform (www.majorbio.com, accessed on 25 July 2025).

2.3.2. Operational Taxonomic Units (OTU)Analysis

To obtain the species classification information of each OTU, RDP (Ribosomal Database Project) classifier was used to analyze the representative sequences of OTU with 97% similarity level, and the species composition of each sample was counted in each taxonomic level: domain, Kingdom, phylum, class, order, family, genus, and species. The taxonomic identity of each phylotype was determined by the bacterial 16S rRNA Silva reference database (http://www.arb-silva.de, accessed on 25 July 2025).

2.3.3. Alpha Diversity Analysis and Rarefaction Curve

Indices of community richness Chao 1 estimator, abundance-based coverage estimator (ACE), and indices of community diversity Shannon, Simpson estimator, were calculated. The coverage (*C*) index reflects whether the sequencing results represent the real situation of microorganisms in the sample. Sobs reflects the observed richness. A certain number of sequences were randomly selected from the samples, and the alpha diversity index of the corresponding samples was counted. Taking the extracted data as the abscissa and the alpha diversity index as the ordinate, the curve was drawn, and whether the sequencing data volume was enough was judged according to whether the curve was smooth or not. In this study, OTUs with 97% similarity were selected to calculate the alpha diversity index under different random sampling by MOTHUR (http://www.mothur.org/, accessed on 25 July 2025), and the curve was made by using the R language tool.

The calculation is based on the following formula [19]:

$$S_{chao1} = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \quad (1)$$

where S_{chao1} represents the estimated number of OTUs, Sobs denotes the actual observed number of OTUs, n_1 indicates the number of OTUs containing only one sequence, and n_2 represents the number of OTUs containing exactly two sequences.

$$S_{\mathrm{ACE}} = \begin{cases} S_{\mathrm{abund}} + \frac{S_{\mathrm{rare}}}{C_{\mathrm{ACE}}} + \frac{n_1}{C_{\mathrm{ACE}}}\hat{\gamma}^2_{\mathrm{ACE}}, \ \hat{\gamma}_{\mathrm{ACE}} < 0.80 \\ S_{\mathrm{abund}} + \frac{S_{\mathrm{rare}}}{C_{\mathrm{ACE}}} + \frac{n_1}{C_{\mathrm{ACE}}}\overset{\sim}{\gamma}^2_{\mathrm{ACE}}, \ \hat{\gamma}_{\mathrm{ACE}} \geq 0.80 \end{cases} \quad (2)$$

where $C_{\mathrm{ACE}} = 1 - \frac{n_1}{N_{\mathrm{rare}}}, N_{\mathrm{rare}} = \sum_{i=1}^{\mathrm{abund}} in_i, \hat{\gamma}^2_{ACE} = max\left[\frac{S_{\mathrm{rare}}}{C_{\mathrm{ACE}}}\frac{\sum_{i=1}^{\mathrm{abund}} i(i-1)n_i}{N_{\mathrm{rare}}(N_{\mathrm{rare}}-1)} - 1, 0\right]$, $\overset{\sim}{\gamma}^2_{\mathrm{ACE}} = max\left[\hat{\gamma}^2_{\mathrm{ACE}}\left\{1 + \frac{N_{\mathrm{rare}}(1-C_{\mathrm{ACE}})\sum_{i=1}^{\mathrm{abund}} i(i-1)n_i}{N_{\mathrm{rare}}(N_{\mathrm{rare}}-C_{\mathrm{ACE}})}\right\}, 0\right]$. n_i represents the number of OTUs containing i sequences, S_{rare} denotes the number of OTUs with 'abund' sequences or fewer than 'abund'; S_{abund} indicates the number of OTUs with more than 'abund' sequences; abund is the threshold for 'dominant' OTUs, defaulting to 10.

$$\mathrm{Shannon} = -\sum_{i=1}^{S_{\mathrm{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N} \quad (3)$$

where n_i represents the number of sequences contained in the i-th OTU, N represents the total number of sequences, and Sobs denotes the actual observed number of OTUs.

$$\mathrm{Simpson} = \frac{\sum_{i=1}^{S_{obs}} n_i(n_i - 1)}{N(N-1)} \quad (4)$$

where symbols and letters in this equation have the same meanings as those in Equation (3).

$$C = 1 - \frac{n_1}{N} \quad (5)$$

where n_1 represents the number of OTUs containing only one sequence; N represents the total number of sequences observed in the sampling.

2.3.4. Statistical Analysis

Principal coordinates analysis (PCoA) was used to evaluate the overall differences in the structures of the plant and bacterial communities based on Bray–Curtis distances. Redundancy analysis (RDA) with Monte Carlo permutation (999 repetitions) was employed to assess the relationships between soil properties and bacterial communities. Correlations between the soil environment factor and composition of the soil bacterial communities were investigated using Pearson's correlation analysis. Heatmap adopts the average level clustering method.

3. Results

3.1. Analysis of Soil Microbial Carbon and Nitrogen

3.1.1. Characteristics of Soil Microbial Carbon and Nitrogen

As shown in Table 2, what was revealed is that the soil microbial C and N content in the 0–100 cm soil layers showed a trend of small change with increasing soil layers. That is, soil microbial activities weaken gradually as the soil depth increases. There is a slight increase in soil microbial C content in the 60–80 cm soil layer of HrN (shady slope of *H. rhamnoides* pure forest), the 80–100 cm soil layer of HrS (sunny slope of *H. rhamnoides* pure forest), the 80–100 cm soil layer of HrBo (*H. rhamnoides* and *Biota orientalis* mixed forest), and the 60–80 cm soil layer of HrPt (*H. rhamnoides* and *Pinus tabulaeformis* mixed forest). There is no clear vertical variation of soil microbial N content, and there are no significant differences between different soil layers. Over the four plots, the soil microbial C content was highest in HrPt, whereas there were few differences in the soil microbial C contents of the other three plots. Soil microbial N content is highest in HrS, and the lowest value is in HrPt.

Table 2. Characteristics of soil microbe carbon and nitrogen in *H. rhamnoides* forests.

Plot Code	Soil Layer (cm)	B_C (mg/kg)	B_N (mg/kg)	B_C/TOC (%)	B_N/TN (%)	B_N/AN (%)
HrN	0–10	135.83	40.01	1.17	3.96	31.02
	10–20	113.42	27.93	1.53	4.67	42.00
	20–40	86.05	16.90	2.05	3.01	29.03
	40–60	59.58	12.95	1.53	3.59	23.67
	60–80	78.15	11.56	3.13	3.23	22.41
	80–100	57.87	7.40	2.89	2.36	17.20
HrS	0–10	157.72	79.00	1.04	6.32	72.48
	10–20	154.14	32.54	1.59	3.97	39.64
	20–40	142.83	20.37	4.08	3.84	32.54
	40–60	100.77	8.45	4.20	2.14	16.64
	60–80	76.73	7.58	3.65	2.22	24.21
	80–100	86.49	6.80	4.12	2.12	22.83
HrBo	0–10	159.40	40.04	0.50	3.15	31.04
	10–20	95.12	28.96	1.29	3.88	37.03
	20–40	82.78	13.22	1.15	1.83	19.88
	40–60	53.73	6.92	0.78	1.12	13.61
	60–80	43.06	5.09	0.67	0.96	9.31
	80–100	59.58	3.10	1.22	0.67	6.60
HrPt	0–10	179.02	18.03	8.95	6.22	65.80
	10–20	127.62	21.17	9.82	9.53	60.13
	20–40	128.60	17.69	6.43	7.76	75.28
	40–60	125.45	29.59	6.27	13.33	54.09
	60–80	175.02	22.54	8.33	10.25	78.82
	80–100	114.30	15.84	8.16	7.27	76.52

3.1.2. Regression Analysis of Soil Microbial Carbon and Nitrogen Contents and Soil Carbon and Nitrogen Contents

Regression analysis, as a method for statistical analysis of the interdependent quantitative relationship between two or more variables, can be used to analyze the relationship between one variable and the change of another variable. The soil microbial C and N content and the soil C and N content in different *H. rhamnoides* forests were analyzed via exponential and linear regression analysis, and we established a regression model. Table 3 shows the results. Apart from the HrPt, the regression equations of the other three samples are well fitted. Of the three well-fitting plots, the R^2 of the index model of soil microbial biomass N and alkali-hydrolysable N of HrS is 0.73, whereas the remainder are all above 0.8, with some even exceeding 0.9. Therefore, the model can well explain the relationship between soil microbial C content and soil organic C content, soil microbial N content and soil total N content, and soil microbial N content and alkali-hydrolyzed N content. Only the soil microbial N content and soil alkali-hydrolyzed N content regression model shows a good fit in the case of HrPt.

3.2. Soil Bacterial Community Diversity

By sequencing the V4–V5 region of bacterial genes, all samples were subsampled to the minimum number of sequences. OTUs with a sequence count of $\geq$5 in at least one sample were retained. A total of 299,625 effective sequences were obtained from the soil bacterial community, with an average length of 438.21 bp. The RDP classifier was used to cluster OTU representative sequences at a 97% similarity level. The results indicated that the bacterial communities comprised 24 phyla, 55 classes, 110 orders, 206 families, 348 genera, 680 species, and 1989 OTUs. By combining OTUs of the same species and

statistically analyzing the changes in species composition among different samples, the diversity of microflora across samples was elucidated.

Table 3. Regression analysis of soil microbial carbon and nitrogen and soil environmental factors in *H. rhamnoides* forests.

Sample Plot Type	Regression Model	B_C and TOC		B_N and TN		B_N and AN	
		Regression Equation	R^2	Regression Equation	R^2	Regression Equation	R^2
HrN	Exponential	$y = 0.7818e^{0.0195x}$	0.83	$y = 0.2493e^{0.0347x}$	0.93	$y = 0.4803e^{0.0219x}$	0.87
	Linear	$y = 0.1103x - 4.4926$	0.88	$y = 0.0205x + 0.134$	0.93	$y = 0.0177x + 0.4375$	0.85
HrS	Exponential	$y = 0.471e^{0.0173x}$	0.92	$y = 0.3343e^{0.0183x}$	0.89	$y = 36.321e^{0.0158x}$	0.73
	Linear	$y = 0.1317x - 10.314$	0.86	$y = 0.0128x + 0.2789$	0.97	$y = 1.0253x + 34.49$	0.87
HrBo	Exponential	$y = 2.5924e^{0.0144x}$	0.84	$y = 0.4803e^{0.0219x}$	0.87	$y = 45.007e^{0.0242x}$	0.94
	Linear	$y = 0.2201x - 7.3738$	0.83	$y = 0.0177x + 0.4375$	0.85	$y = 1.9374x + 39.59$	0.90
HrPt	Exponential	$y = 0.9366e^{0.0045x}$	0.35	$y = 0.2637e^{-0.006x}$	0.08	$y = 7.5664e^{0.0662x}$	0.90
	Linear	$y = 0.0076x + 0.7217$	0.36	$y = -0.0016x + 0.2659$	0.08	$y = 2.3702x - 17.641$	0.91

Table 4 presents the sequence number of the test samples and the alpha diversity index of the microbial community. The coverage of each sample is greater than 98%, which indicates that the probability of gene sequence detection in soil samples is very high, and the sampling is basically reasonable, which can truly reflect the microbial community characteristics of soil samples. According to the rarefaction curves of each diversity index (Figure 1), with the increase in random sequencing data, the dilution curve basically tends to be flat, which indicates that the confidence degree of microbial community in the real environment is high, and the sequencing depth and data volume are enough. What is more, the richness indices and diversity indices of the bacterial community in arbor shrub mixed forest are higher than those in shrub pure forest, and the indices of shrub forest on sunny slope are higher than those on shady slope (Table 4).

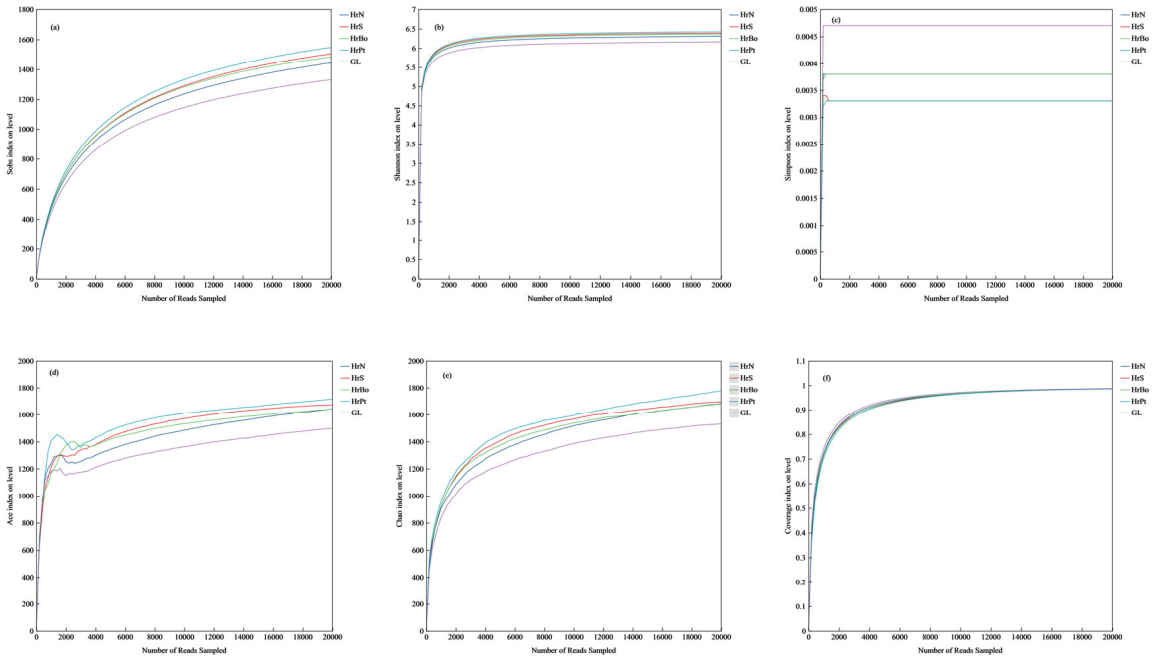

Figure 1. Rarefaction curves at cutoff level of 3%. (**a**) Sobs curves, (**b**) Shannon curves, (**c**) Simpson curves, (**d**) Ace curves, (**e**) Chao1 curves, (**f**) Coverage curves.

Table 4. Sequence statistics and diversity index of the bacterial communities.

Plot Code	Mean Length	Sobs	Shannon	Simpson	Ace	Chao1	Coverage
HrN	438.57 ± 0.89 a	1398 ± 72 a	6.20 ± 0.11 a	0.004 ± 0.001 a	1596.33 ± 73.19 a	1632.10 ± 65.23 a	0.986 ± 0.000 bc
HrS	439.01 ± 0.34 a	1428 ± 149 a	6.27 ± 0.19 a	0.004 ± 0.001 a	1619.04 ± 49.26 a	1633.50 ± 15.69 a	0.986 ± 0.001 ac
HrBo	438.48 ± 0.68 a	1486 ± 23 a	6.35 ± 0.01 a	0.003 ± 0.000 a	1653.70 ± 12.35 a	1681.85 ± 18.58 a	0.987 ± 0.001 ac
HrPt	438.15 ± 0.77 ab	1500 ± 69 a	6.37 ± 0.09 a	0.003 ± 0.001 a	1660.69 ± 79.06 a	1683.02 ± 11.59 a	0.987 ± 0.001 ac
GL	436.81 ± 0.92 a	1394 ± 64 a	6.19 ± 0.15 a	0.005 ± 0.002 a	1559.06 ± 56.21 a	1577.40 ± 49.96 a	0.987 ± 0.000 a

Note: Values are means ± standard error (n = 3). Different letters indicate significant differences ($p < 0.05$) among soils for the individual variables based on a one-way ANOVA followed by an LSD test.

3.3. Soil Bacterial Community Structure

The dominant phylum in the bacterial community is Actinobacteria (37.27% on average), followed by Proteobacteria (23.91%), Acidobacteria (12.75%), Chloroflexi (12.27%), Gemmatimonadetes (5.85%), Nitrospirae (1.66%), Bacteroidetes (1.26%), GAL15 (1.14%), and Firmicutes (1.1%) (Figure 2). There are differences in the relative abundance of the dominant phylum between the pure and mixed artificial forests, but the differences are not significant. Among the Proteobacteria, Alpha-, Beta-, Gamma-, and Deltaproteobacteria are found in all samples (Figure 3). The members of Alphaproteobacteria dominate this phylum, occupying 14.67% of all populations, the highest at GL compared to the others. Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria comprise 4.30%, 2.75%, and 2.19% of the total populations, respectively.

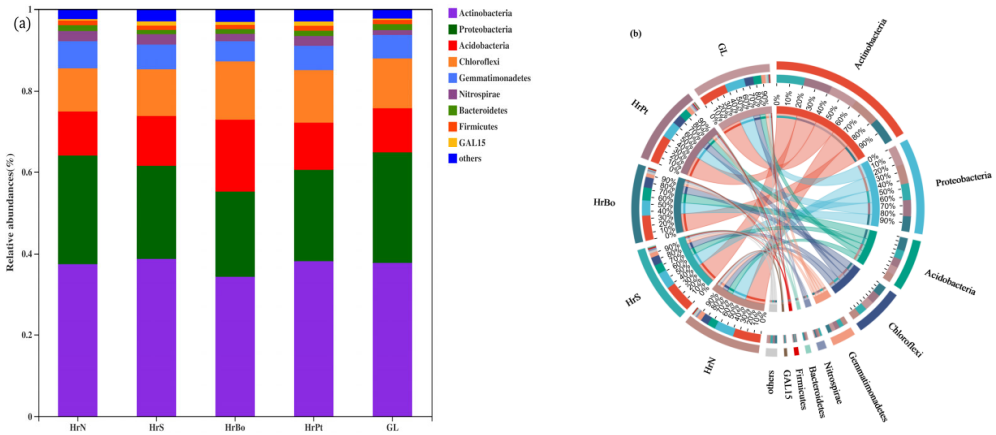

Figure 2. Relative abundance (**a**) and Circos (**b**) of bacterial community at the phylum level. Others: Relative abundance <1% in all samples.

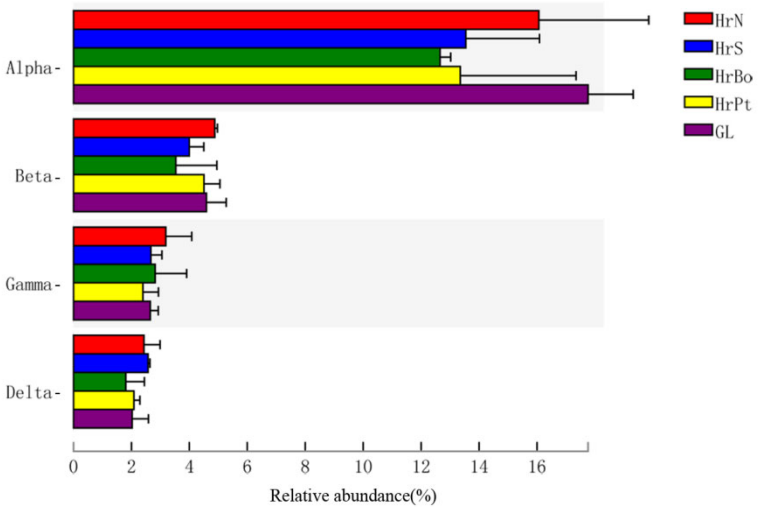

Figure 3. Distribution of Proteobacteria populations at the class level.

The plot of PCoA (Figure 4) clearly identifies variations in bacterial community composition among the sites, with the abundance of each group varying with different afforestation modes. At the OTU level, the interpretation variance of PC1 and PC2 is 24.46% and 19.37%, respectively, and the accumulated interpretation ability is 43.83%. The profiles at HrN and HrBo sites tend to group together and are clearly separated from those at GL, HrS, and HrPt sites, which are clearly separated from each other.

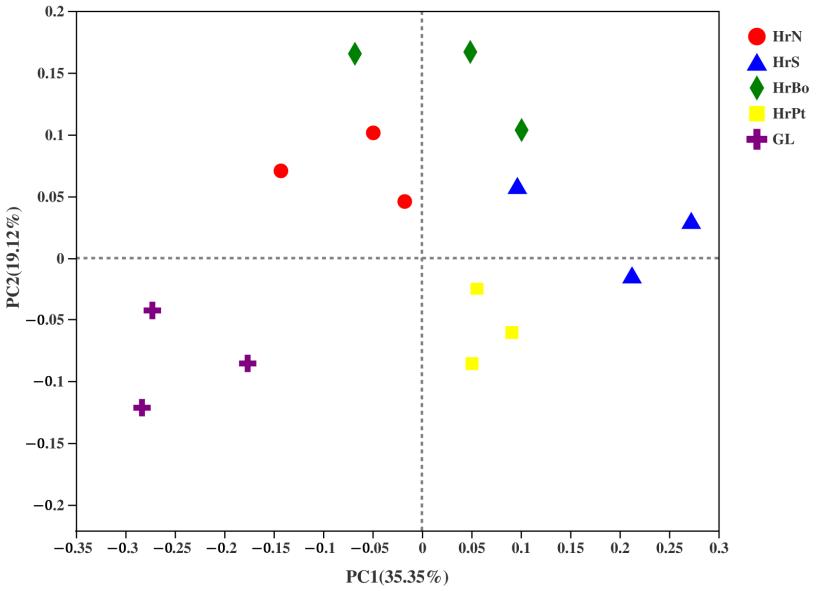

Figure 4. Principal coordinates analysis (PCoA) of bacterial composition based on Bray–Curtis distances.

3.4. Relationships Among Soil Properties Factor and Bacterial Communities

The Pearson correlation coefficient was used to analyze the correlation between bacterial communities and soil environmental factors. The results of the analysis are illustrated by a heatmap in Figure 5. TOC, AN, AP, TN, and AK contents are correlated positively with the abundance of Alphaproteobacteria and Acidobacteria and negatively with the abundance of Gemmatimonadetes and Bateproteobacteria. AP and AK are correlated negatively with the relative abundance of Nitrospira.

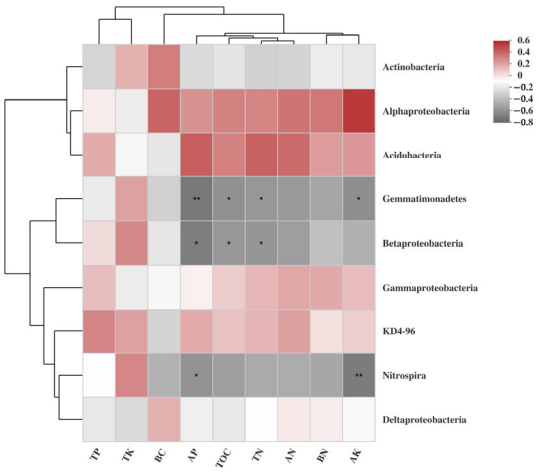

Figure 5. Heatmap of bacterial and soil properties factors. R and *p* values are obtained by calculation. The R value is shown in different colors in the graph. The color card on the right-hand side of the heatmap is a color partition with different R values. $0.01 < p \leq 0.05$ *, $0.001 < p \leq 0.01$ **. BC: microbial biomass C, BN: microbial biomass N, TOC: total organic C, TN: total N, AN: alkali-hydro N, TP: total P, AP: available K, TK: total K, AK: available K, the same as follows.

The RDA confirmed the results of the correlation analysis and further identified the effect of the soil on the bacterial communities at the class level (Figure 6). The first two axes explain 87.77% of the total variance, indicating that TOC, TN, AN, AP, and AK were the most influential factors driving the changes in the composition and diversity of the bacterial communities.

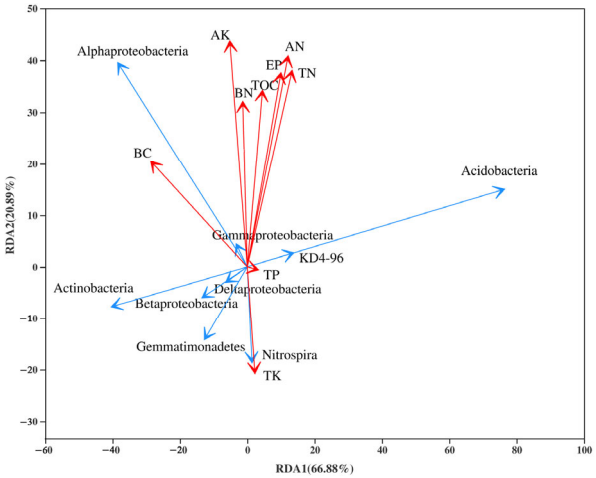

Figure 6. Ordination plots of the results from the redundancy analysis to identify the relationships among the bacterial populations (blue arrows) and soil properties factors (red arrows).

4. Discussion

Restoration mainly affects the quantity and community of soil microorganisms by affecting the litter, root morphology, and secretion, and the material and energy transformation process in the whole ecosystem [6]. *Hippophae rhamnoides*, which is a pioneer and associated species of vegetation structure in the Loess Plateau, has clear ecological and economic benefits. Its root system can co-exist with nitrogen (N)-fixing actinomycetes to form N-fixing nodules and fix free N. Furthermore, this root system can decompose insoluble organic matter and minerals, thereby helping to improve the soil [20].

Based on the analysis of microbial biomass C and N in typical artificial forests, the driving capacity of the soil carbon (C) cycle and the mineralization and fixation of soil microorganisms on nitrogen (N) were examined. The soil microbial C and N analysis showed that soil microbial biomass N and N in different forest layers exhibited a trend of a small change in the 0–100 cm soil layers; that is, as the soil depth increased, the soil microbial activity weakened gradually. Studies indicate that changes in soil microbial biomass C and N content are likely attributed to shifts in microbial community composition. Furthermore, soil enzyme activity significantly influences the characteristics of microbial biomass. Wu et al. [21] studied the variation rules of nutrient limitation dynamics in different vegetation restoration types in the Loess Plateau, and the results showed that mixed forests demonstrated higher enzyme activity and nutrient content compared to pure forests. Cui et al. [22] investigated the changes in soil microbial community structure and their response to nutrient limitations in the ecotone of the desert steppe on the Loess Plateau. There were significant differences in soil microbial biomass carbon and nitrogen contents among different vegetation types. From south to north on the Loess Plateau, the microbial biomass carbon and nitrogen contents showed a significant decrease. The soil microbial biomass follows this pattern: natural grassland > artificial shrub and woodland > farmland [22]. They also showed that, in terms of increasing soil microbial biomass carbon and nitrogen content, the mixed mode of forest and grass was more effective than the single vegetation and could effectively promote soil remediation. Therefore, it is suggested that the mixed forest should be the main way to implement the Grain for Green Project in the Loess Plateau.

Soil microbial diversity is influenced by various environmental factors, which not only affect the diversity of soil microorganisms but also impact the structure, function, and processes of soil ecosystems. There are differing views on the factors influencing the composition of soil microbial communities. Zhang et al. [23] suggested that the composition of soil microbial communities is mainly driven by external environmental conditions, while Ding [24] and Chen et al. [25] argued that soil properties play a crucial role. In this study, we selected afforested plots under the same climatic conditions to analyze the impact of basic soil properties on the dominant phyla of soil bacterial communities. Studies have shown that Actinobacteria are the main functional bacteria that degrade lignin and cellulose [26]. Alpha-, Beta-, Gamma-, and Deltaproteobacteria are the most important microorganisms in saline alkali soil [27]. In this study, we found that Actinobacteria and Proteobacteria had relative abundances of 37.23% and 23.91%, respectively, making them the most dominant bacteria in the community. These findings are consistent with research on the characteristics of soil bacterial communities in different forests in the Loess Plateau, and the vegetation type was an important factor affecting the diversity of soil Actinobacteria [28]. TOC, TN, AN, AP, and AK were the most influential factors driving the changes in the composition and diversity of the bacterial communities. Xu et al. [29] reported the responses of soil *nosZ*-type denitrifying microbial communities to the various land-use types of the Loess Plateau, and the results showed that AN was the key environmental factor affecting microbial composition. Gao et al. [30] reported the changes in soil microbial community structure following different tree species functional traits afforestation, and the results showed that the microbial structures associated with soil carbon and nitrogen were significantly increased.

This study only addresses the soil microbial characteristics of different stands in the growing season during revegetation in Wuqi City, Shaanxi Province. To better analyze the mechanism of influence of vegetation on microorganisms and the influence of site conditions and environmental factors on soil microbial community diversity, the litter and root exudates under various artificial forests can be studied further. To better analyze the microbial characteristics under the effect of revegetation in the Loess Plateau, similar studies can be conducted in other areas of the Loess Plateau. The results of these experiments can be analyzed to better explore the soil under *H. rhamnoides* forest in the process of vegetation diversity of soil microbial community, and are expected to provide some data supporting the study of revegetation in the Loess Plateau.

5. Conclusions

In this study, the soil bacteria were analyzed as a function of different vegetation restoration types on the Loess Plateau. Soil microbial biomass C and N in different forest layers exhibited a trend of a small change in 0–100 cm soil layers; that is, as the soil depth increases, the soil microbial activity weakens gradually. The richness indices and diversity indices of the bacterial community in arbor shrub mixed forest are higher than those in shrub pure forest, and the indices of shrub forest on sunny slope are higher than those on shady slope. Across all samples, the dominant groups were Actinobacteria (37.27% on average), followed by Proteobacteria (23.91%), Acidobacteria (12.75%), and Chloroflexi (12.27%). Soil nutrient supply, such as TOC, TN, AN, AP, and AK, has crucial roles in shaping the composition and diversity of the bacterial communities. In future research, we can further explore the dynamic process and mechanisms of changes in soil microbial community structure during vegetation restoration, as well as how to optimize soil microbial community structure through the regulation of vegetation restoration measures to enhance soil quality and ecological functions. This will help us better understand the impact of

vegetation restoration on soil ecosystems, providing a scientific basis for environmental protection and sustainable development.

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Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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