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Soil Microbial Communities in *Pseudotsuga sinensis* Forests with Different Degrees of Rocky Desertification in the Karst Region, Southwest China

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Abstract: Rocky desertification (RD), a natural and human-induced process of land degradation in karst areas, has become the primary ecological disaster and one of the obstacles to sustainable ecological development in southwest China. Nevertheless, the variation of soil physical and chemical properties, bacterial and fungal communities, and their relationships in RD forests remains limited. Therefore, soil samples were collected from forests under four degrees of RD (NRD, non-RD; LRD, light RD; MRD, moderate RD; and SRD, severe RD) and subjected to high-throughput sequencing of 16S rRNA and ITS1 genes. The results showed a significant reduction in bacterial richness and diversity, while fungal richness and diversity decreased markedly and then showed a balanced trend with the increase in RD degree, indicating that bacteria and fungi did not present the same dynamics in response to the process of RD. The bacterial communities were dominated by Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi, while the fungal communities were dominated by Basidiomycota, Ascomycota, and Mortierellomycota. The PCoA and NMDS demonstrated significant differences in microbial communities in study sites, among which the fungal communities in non-RD forest and LRD forest clustered together, suggesting that fungal communities were more stable than bacteria in RD forest. The db-RDA, Mantel test, and random forest model confirmed the important role of soil BD, pH, SOC, AN, and AP in driving microbial diversity and communities. The IndVal analysis suggested that Chloroflexi, Patescibacteria, Atheliales, and Cantharellales with high indicator values were identified as potential bio-indicators for RD forests. This study could not only improve our understanding of bacterial and fungal community dynamics across RD gradients, but also could provide useful information for the further use of microorganisms as indicators to reflect the environmental changes and ecosystem status during forest RD.

Keywords: karst area; rocky desertification; soil properties; microbial community; bio-indicators

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1. Introduction

Karst refers to a special landform formed by the dissolution and transformation of carbonate rocks by groundwater and surface water under special geological conditions, and is widely distributed globally [1]. Karst covers an area of approximately 5.1×10^7 km^2 globally, accounting for about 12% of the world's land area, mainly distributed in regions such as the Mediterranean, Eastern Europe, the Middle East, Southeast Asia, and the Caribbean [1]. China is one of the world's three major concentrated karst distribution areas, with a karst area of over 1.3 million km^2, accounting for 13.54% of the total land area [2].

Within this area, the southwestern region of China, mainly in Guizhou, has the largest and most concentrated contiguous karst ecological fragile area in Asia, with a karst area of 550,000 km^2, accounting for over 70% of the total land area of the province [3]. This region is not only the area with the widest and most developed distribution of tropical and subtropical karst in the world, but also the most typical and representative region for karst environmental and ecological issues. Due to the unique geological structure of karst and the long-term shaping by climatic and hydrological factors, the spatial distribution of water and soil resources is uneven, and the hydrothermal conditions exhibit high spatiotemporal heterogeneity. Moreover, population explosion, severe soil erosion, frequent natural disasters, and ecosystem vulnerability exacerbate the deterioration of the ecological environment, severely affecting the local people's living standards and hindering the social and economic development in the area [4].

Rocky desertification (RD) refers to a land degradation process occurring in the vulnerable karst environment of the subtropical regions, resulting from human-induced disturbances [5]. It leads to severe soil erosion, extensive exposure of bedrock, a significant decline in land productivity, and the appearance of desert-like landscapes on the surface. In recent years, with the rapid increase in population, the issue of RD has become increasingly severe and has emerged as a global environmental problem [6]. As one of the largest and most typical continuous karst distribution belts in the world, southwest China has the largest and most typical distribution of RD, with an annual growth rate of 0.91% [7]. The southwestern karst region, represented by Guizhou, is the central area of karst development in East Asia. Due to its lower environmental carrying capacity and significant human–land conflicts, a series of ecological degradation phenomena, such as soil erosion, vegetation destruction, and exposure of bedrock, are particularly common in this region, leading to the formation of RD landscapes. As the intensity of RD intensifies, soil erosion worsens, and the soil's capacity for nutrient retention, supply, and fertility rapidly diminishes, even leading to the loss of land productivity. RD has become one of the environmental challenges that restrict large-scale development and a significant ecological problem for agricultural production and economic development in China. Previous studies indicated that RD leads to changes in soil properties, a decline in plant diversity, and reductions in both above- and below-ground biomass [4]. Despite these findings, there is a significant gap in our understanding of how soil microbial composition and diversity are affected by RD in karst areas.

Soil microorganisms play a crucial role in the functioning and services of soil ecosystems, such as plant productivity, nutrient cycling, and organic matter decomposition [8]. They are essential components of the soil environment and regulate ecosystem nutrient cycling through their metabolic activities and interactions with other biotic and abiotic factors. Due to their short life cycles and sensitivity to environmental changes, soil microorganisms can respond rapidly to ecological and environmental perturbations, making them sensitive indicators of the soil environment [9]. The diversity of soil microorganisms is regulated by various factors, including both natural and anthropogenic influences. The importance of different factors in controlling soil microbial communities depends on the spatial scale. At the global and regional scales, microbial communities are more influenced by climatic factors, soil environment, and nutrients, such as soil pH and organic carbon content [10,11]. On the other hand, at local or smaller micro-scales, factors such as plant characteristics, soil microenvironment, interactions between trophic levels in the soil food web, and inter-species interactions among microorganisms might become more important in controlling microbial communities [12].

In the karst region, the ecological structure is fragile, and microorganisms actively participate in the weathering of karst rocks (limestone). They actively accelerate the dissolution of carbonate rocks. Studies have found the presence of autotrophic nitrogen-fixing microorganisms and autotrophic photosynthetic microorganisms on karst rocks undergoing autotrophic weathering [13]. Moreover, heterotrophic microorganisms are also found on autotrophically weathered rocks, and under favorable conditions, they can

proliferate and spread rapidly, thereby accelerating the weathering of carbonate rocks to some extent [14]. The process of RD affects soil properties and vegetation community characteristics. As the degree of RD increases, the content of soil organic matter and plant biomass declines. RD has different influences on soil bacterial community composition, while the richness and diversity of bacterial communities do not show significant changes with the increase in RD [15]. In fact, the bacterial diversity slightly increases and the richness slightly decreases in severe RD areas. Moreover, in heavy RD areas, the soil fungal community differs significantly from other degrees of RD, with soil total nitrogen being the main factor correlated with fungal communities, followed by pH [16]. The phylum Ascomycota is the most abundant in non-degraded soils, while Basidiomycota dominates in severe RD soils, and the ratio of Ascomycota to Basidiomycota decreases significantly with the deepening of RD, which can serve as an indicator of the degree of RD [16]. In the degraded karst ecosystems, a study has shown that soil degradation in the karst region reduced soil bacterial genetic diversity and significantly changed community structure [17]. However, the dynamics of diversity and compositions of soil bacterial and fungal communities in response to RD, and their driving factors, are still unclear.

Furthermore, effective ecosystem management and restoration strategies require sensitive indicators to assess the state of the ecosystem. Soil microbes play a critical role in community reconstruction, serving as valuable indicators of ecosystem conditions [18]. Firstly, microorganisms represent the largest component of total soil biomass and are vital drivers of processes essential for ecosystem services, such as organic matter decomposition, biodiversity, and plant productivity [19]. Secondly, microorganisms respond rapidly to environmental conditions due to their high surface-to-volume ratio and intimate relationship with their surroundings. This rapid responsiveness makes them valuable indicators of environmental change. Thirdly, molecular methods offer a convenient way to track changes in microbial diversity and community composition, translating them into tangible parameters. Therefore, deriving parameters from microbial diversity, community composition, and sensitive taxa to degraded ecosystem offers a promising approach to evaluate ecosystem conditions. Thus, recognizing the importance of microbial indicators in evaluating habitat status, further exploration of microbial evaluation strategies utilizing high-throughput sequencing technologies is crucial.

In this study, soil physicochemical properties were determined and high-throughput sequencing technology was used to sequence the soil bacteria (16S rRNA) and fungi (ITS1) to assess the relationships between soil properties and microbial communities in forests under different degrees of RD in the karst region. The main purposes of this study are (i) to determine the diversity and taxonomic compositions of bacterial and fungal communities across the RD gradient, (ii) to uncover the key factors driving bacterial and fungal communities in karst area, and (iii) to identify the potential microbial taxa as bioindicators of the degrees of RD.

2. Materials and Methods

2.1. Study Site and Sampling

The study area is in Weining *Pseudotsuga sinensis* Nature Reserve of Bijie City, Guizhou province, southwest China (103.93–104.26° E, 26.54–26.76° N), with elevations ranging from 1800 to 2450 m, covering a total area of 951.86 hectares (Figure 1). Before the nature reserve was established, the study region faced intensifying anthropogenic disturbances, such as agricultural cultivation and logging, which inflicted destructive impacts on the already fragile forest, compromising its regenerative capacity and competitive survival. This led to severe soil erosion, exacerbating the process of rocky desertification. To sensibly protect biological resources and restore degraded karst habitats, the People's Government of Weining County officially approved the establishment of a county-level nature reserve in August 2000, aiming to prevent further degradation of the karst habitat. In order to further enhance the efforts in ecosystem conservation, the government undertook further optimization of the nature reserve in March 2022. The reserve experiences a warm and

humid subtropical monsoon climate, with an average annual temperature of 10.5 °C and an annual average rainfall of 1000 mm, of which more than 70% occurs during the time from June to September. The predominant soil type in this area is Leptosols, which are soils limited by hard rock or have a low percentage of fine earth material according to World Reference Base for Soil Resources (WRB). The region boasts rich and well-preserved vegetation resources, primarily consisting of warm-temperate coniferous forests dominated by *P. sinensis* and *Pinus yunnanensis* Franch. The shrublands are mainly composed of *Cotoneaster franchetii* Bois., *Corylus yunnanensis*, *Viburnum dilatatum* Thunb., *Hypericum monogynum* L., and *Coriaria napalensis* Wall.

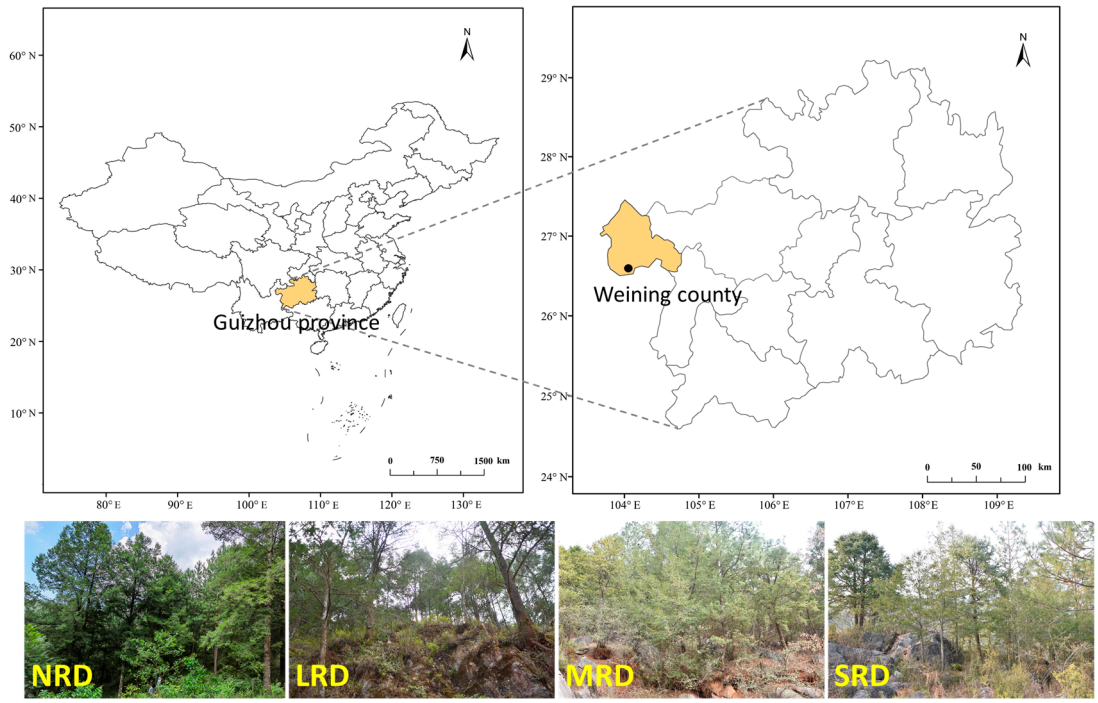

Figure 1. Location of the study area and the pictures of four degrees of rocky desertification in Guizhou Province. NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification.

In June 2022, four regions with different degrees of rocky desertification (NRD: non-rocky desertification, LRD: light rocky desertification, MRD: moderate rocky desertification and SRD: severe rocky desertification) were selected according to the industry standard of the State Forestry Administration (LY/T 1840-2009) [20] and the classification standard of rocky desertification [21]. The soil depth in NRD, LRD, MRD, and SRD forests were approximately 50–60 cm, 30–40 cm, 20 cm, and 10 cm, respectively. Moreover, the bare rock rate in NRD, LRD, MRD, and SRD forests were approximately 20%–30%, 40%–50%, 70%, and 80%, respectively, based on visual assessment. Then, six plots of 50 × 50 m were set up within each region with different degrees of rocky desertification. The distance between each plot was at least 50 m. After removing surface litter, soil samples were collected from the 0–10 cm soil layer using a shovel and every sample was a mixture of three subsamples collected in an "S" shape. These soil samples were then passed through a 2 mm sieve to remove impurities such as stones and plant residues. The ring knife was inserted into the ground to obtain a soil core. The sieved soil samples were divided into two portions. The first potion (~5 g of soil) was preserved in a cooler and transported to the laboratory within 24 h. These samples were stored at −80 °C and used for the extraction of total soil DNA. The second potion (~200 g of soil) was stored in self-sealing bags, transported to the laboratory, and air dried naturally. After drying, the soil was ground and passed through a 100-mesh sieve. These samples were used for the determination of soil properties,

including soil pH, soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), total potassium (TK), and available potassium (AK). The third potion (soil core) was then placed in a sterile plastic bag for the measurement of soil bulk density (BD).

2.2. Soil Properties

Soil BD was measured via the ring knife method. Specifically, the metal ring was pressed into the soil (intact core), and then the soil samples were placed into a sterile plastic box to preserve for lab drying. The soil inside the metal ring was dried to constant weight at a high temperature of 105 °C (for at least 24 h) and weighed to calculate soil BD [22]. Weighed soil samples (20 g fresh weight) were made into a soil suspension via the addition of deionized water in a soil:water ratio of 1:5. Soil pH was measured using a FE28-Standard pH meter (Mettler-Toledo, CA, USA) [23]. SOC was determined via potassium dichromate oxidation with external heating [23]. Specifically, 0.5 g of the dry soil samples was weighed in a 250 mL Erlenmeyer flask containing 10 mL of K_2Cr_2O7 (0.8 M) and 10 mL of H_2SO_4, and then boiled at 170–180 °C for 5 min. TP was determined using the molybdate colorimetric method after perchloric acid digestion and ascorbic acid reduction [24]. AP was measured via extraction with 0.5 M $NaHCO_3$ (pH = 8.5) for 30 min and then assessed colorimetrically via the molybdate-ascorbic acid method using a UV spectrophotometer [25]. TN was determined via Kjeldahl digestion [26]. Specifically, 0.5 g soil was digested with 5 mL of concentrated H_2SO_4. The digestion was initially started at 50 °C and then raised gradually to 350 °C. The samples were analyzed using 40%NaOH solution, and 4% boric acid, 0.1 N standard HCl solutions. AN were qualified according to the dichromate oxidation [26]. TK in soil was determined via NaOH alkali fusion-flame photometry, while AK was extracted with 1 M ammonium acetate ($CH_3CO_2NH_4$) at pH 7.0 and subsequently measured using flame photometry [27].

2.3. DNA Extraction, Illumina Sequencing, and Sequencing Data Processing

The total microbial genomic DNA in the soil samples was extracted from 0.5 g of frozen soil using a PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's recommendations. Detailed information on primer sets, PCR conditions, and reaction systems for bacterial 16S rRNA and fungal ITS1 genes are shown in Table S1. The high-throughput sequencing was performed by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) based on an Illumina MiSeq platform (San Diego, CA, USA). Bioinformatic processing of sequencing data was conducted with QIIME 2 2019.4 with modifications according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/, accessed on 10 October 2022) (Supporting Information, Method 1). The Illumina sequencing raw read data deposited in the Sequence Read Archive (SRA) are available in the NCBI SRA portal with PRJNA797899, BioProject ID.

2.4. Statistical Analysis

After confirmation of the homogeneity of variances and normality of the data using the Levene and Shapiro–Wilk tests, respectively, one-way analysis of variance (ANOVA) was used to determine the differences in soil physicochemical properties, Bray–Curtis dissimilarities, richness (Sobs index and Chao1 index) and α-diversity (Shannon index) of microbial communities in different forests under different degrees of RD via SPSS (version 21.0, Chicago, IL, USA). The richness (Sobs index, and Chao1 index) and α-diversity (Shannon index) of bacterial and fungal communities were performed by the QIIME2 software (version 2019.4). Sobs was the number of species observed in the sample. The formulas used for the calculation of Chao1 and Shannon indices were as follows:

$$\text{Chao1} = \text{Sobs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \quad (1)$$

$$\text{Shannon} = -\sum_{i=1}^{s} p_i(lnp_i) \quad (2)$$

where Sobs is the number of ASVs observed, n_1 is the number of OTUs with only one sequence, and n_2 is the number of ASVs with only two sequences. p_i is the proportion of individuals belonging to *i*-species in the sample.

The Venn and upset Venn diagrams were performed using the "VennDiagram" and "UpSetR" packages, respectively, to show the unique and shared amplicon sequencing variants (ASVs) in each study site. Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) were used to explore the structural variation of bacterial and fungal communities in forests under different degrees of RD based on Bray–Curtis distance using the "Vegan" package. Similarly, the β-diversity of bacterial and fungal communities in each study site was calculated based on the Bray–Curtis dissimilarity index using the "Vegan" package. The indicator value (IV) index was derived based on the relative abundance and relative frequency of occurrence to identify bio-indicators associated with each degree of RD using the "IndVal" package. Distance-based redundancy analysis (db-RDA) and Mantel tests were used to explore the correlations within the microbial communities in the context of soil properties based on relative abundances of ASVs using "vegan" and "LinKET" packages, respectively. Pearson's correlation coefficient was used to measure the relationships between the diversity of microbial communities and soil properties. Subsequently, random forest analysis was performed to determine the importance of each soil parameter for the α-diversity and β-diversity of bacterial and fungal communities based on MSE using the "randomforest" package (version 4.7-1.1) [28].

3. Results

3.1. Soil Properties in Different Study Sites

During the process of rocky desertification (RD), the soil's physical and chemical properties changed dramatically among different study sites (Figure 2). Compared with the control, i.e., NRD forest (1.01 ± 0.10 g cm^{-3}), soil bulk density (BD) significantly decreased in the MRD (1.14 ± 0.09 g cm^{-3}) and SRD forests (1.35 ± 0.06 g cm^{-3}), but did not change in the LRD forest (1.10 ± 0.12 g cm^{-3}). NRD and LRD forests had lower soil pH than MRD and SRD, but had no difference between them. The soil organic carbon (SOC) decreased significantly, from 53.19 ± 3.57 g kg^{-1} (NRD) to 49.79 ± 2.11 g kg^{-1} (MRD) and 40.39 ± 4.13 g kg^{-1} (SRD), but did not change in the LRD forest (55.65 ± 4.07 g kg^{-1}). Soil total nitrogen (TN) and total phosphorus (TP) did not change significantly among different study sites. Compared with NRD and MRD forests, soil total potassium (TK) content in LRD and SRD forests was significantly increased, and the change between LRD and SRD forests was not significant. The soil available nitrogen (AN) and available phosphorus (AP) showed significant variations among different study sites, with the AN content ranking, from high to low, as LRD > NRD > MRD > SRD, while the AP content ranking was LRD > NRD > SRD > MRD. Compared with the MRD forest, NRD, LRD, and SRD forests had significantly higher available nitrogen potassium content, while no difference in available potassium (AK) was found among the three study sites.

3.2. Sequencing Information and α-Diversity of Microbial Communities

A total of 1,960,415 and 2,114,212 reads were obtained for 16S V3-V4 and ITS1 datasets, respectively. The DADA2 pipeline allowed identification of 110,692 and 10,728 bacterial and fungal ASVs, respectively after removing singletons. Bacterial ASVs represented 36 phyla, 118 classes, 311 orders, 525 families, 1105 genera, and 1643 species, while fungal ASVs represented 16 phyla, 51 classes, 127 orders, 278 families, 601 genera, and 959 species. All rarefaction curves were saturated with increased sequencing amounts (Figure 3a,b) and the coverage exceeded 99%, which indicated that most soil bacteria and fungi were sequenced in all samples.

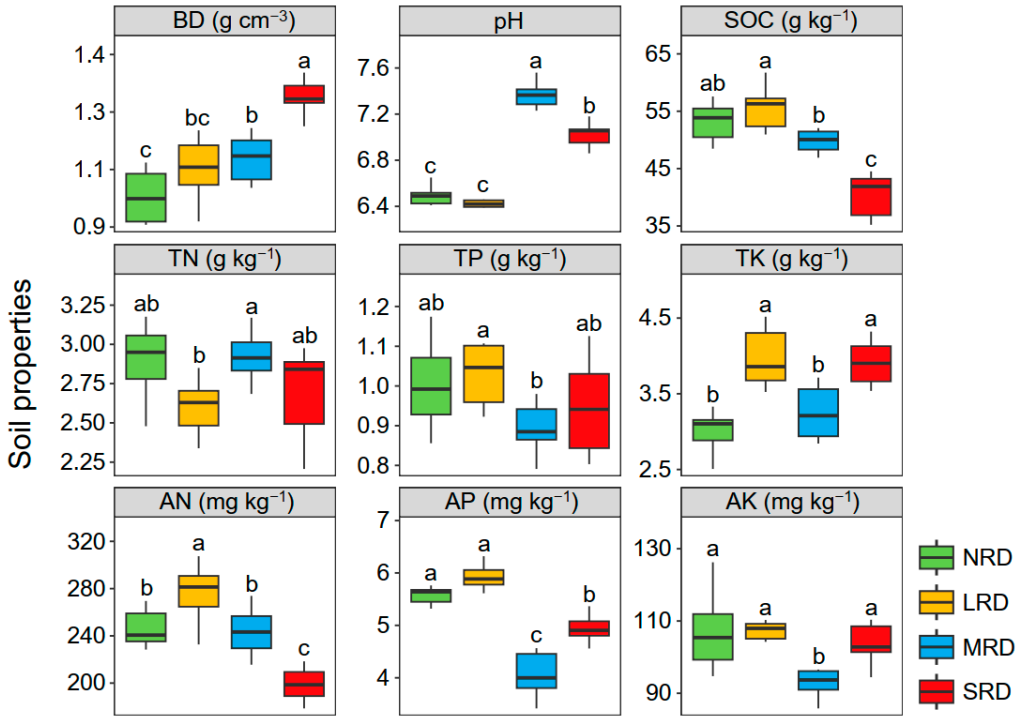

Figure 2. Variations in soil properties in study sites with different degrees of rocky desertification. Different letters indicate a significant difference at $p < 0.05$ according to Duncan's multiple range test. NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification. BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; and AK, available potassium.

In the current study, observed species (Sobs), Chao1, and Shannon indices were used to evaluate the alpha-diversity of soil microbial communities (Figure 3c–h). The results showed that RD had a strongly negative impact on the α-diversity of soil bacterial communities. NRD forest had the highest level of bacterial community richness. The Sobs, Chao1, and Shannon indices of bacterial communities in the SRD forest decreased significantly when compared with those in the NRD forest ($p < 0.05$). However, no difference was found in bacterial α-diversity between MRD and SRD forests. In terms of fungal α-diversity, the NRD forest showed the highest values of Sobs and Chao1 indices, while there was no difference among LRD, MRD, and SRD forests. The Shannon index of fungal communities in the NRD forest was higher compared with that in the MRD forest. Bacterial α-diversity was considerably higher than fungal diversity, e.g., there were approximately ten times as many bacterial ASVs as there were fungal ASVs.

3.3. Composition of Microbial Communities

As shown in Figure 4, the bacterial phyla Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi accounted for the largest proportion in all soil samples, with percentages of 30.16%, 17.87%, and 16.58%, respectively (Figure 3a). By contrast, fungal phylum Basidiomycota, Ascomycota, and Mortierellomycota accounted for the largest proportion in all soil samples, with percentages of 64.33%, 27.84%, and 1.44%, respectively. Moreover, there were considerable differences in microbial community composition along the forests with different degrees of rocky desertification. For instance, Actinobacteria accounted for 34.23% of bacterial community composition in NRD forests but only 15.5% and 15.8% in MRD and SRD forests, respectively; moreover, Proteobacteria and Chloroflexi only accounted for 27.71% and 10.55%, respectively, of bacterial community composition in NRD forest but 34.15% and 23.72% in SRD forest. Soil bacterial and fungal phyla had

different sensitivity and adaptability to RD. In this study, varying kinds of changes were found in the insensitive and sensitive phyla with the increase in RD gradient. For instance, Gemmatimonadetes, Verrucomicrobia, Rokubacteria, and Planctomycetes showed "arch" changes, whereas Ascomycota, Mortierellomycota, Rozellomycota, Zoopagomycota, and Glomeromycota showed "inverted arch" changes.

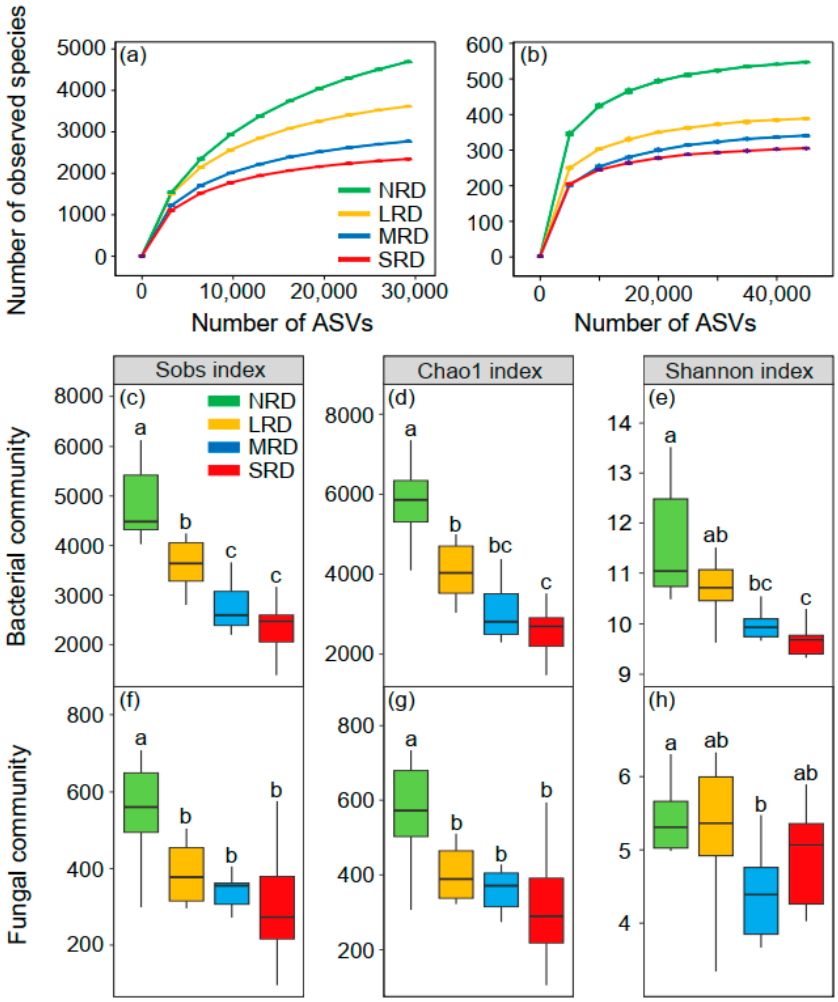

Figure 3. Rarefaction curves and alpha-diversity plots of soil microbial communities along four degrees of rocky desertification. Rarefaction curve constructed based on observed ASVs and species of bacterial (**a**) and fungal (**b**) communities. Alpha-diversity is assessed by richness (Sobs and Chao1 indices) and diversity (Shannon index) of bacterial (**c**–**e**) and fungal (**f**–**h**) communities. Different letters indicate a significant difference at $p < 0.05$ according to Duncan's multiple range test. NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification.

The Venn diagrams showed the ASV level for the microbial community of soil samples (Figure 4c,d). The number of unique fungal ASVs decreased with increasing degrees of rocky desertification: the highest number was found in the NRD forest (1990), followed by the LRD forest (1148) and MRD forest (931), while the SRD forest had the lowest number (868). However, the distribution of unique bacterial ASVs was different compared with that of unique fungal ASVs. The number of unique bacterial ASVs increased firstly and then decreased: the highest number was found in the LRD forest (19,376), followed by the NRD forest (13,372) and MRD forest (8849), while the SRD forest had the lowest number (7158).

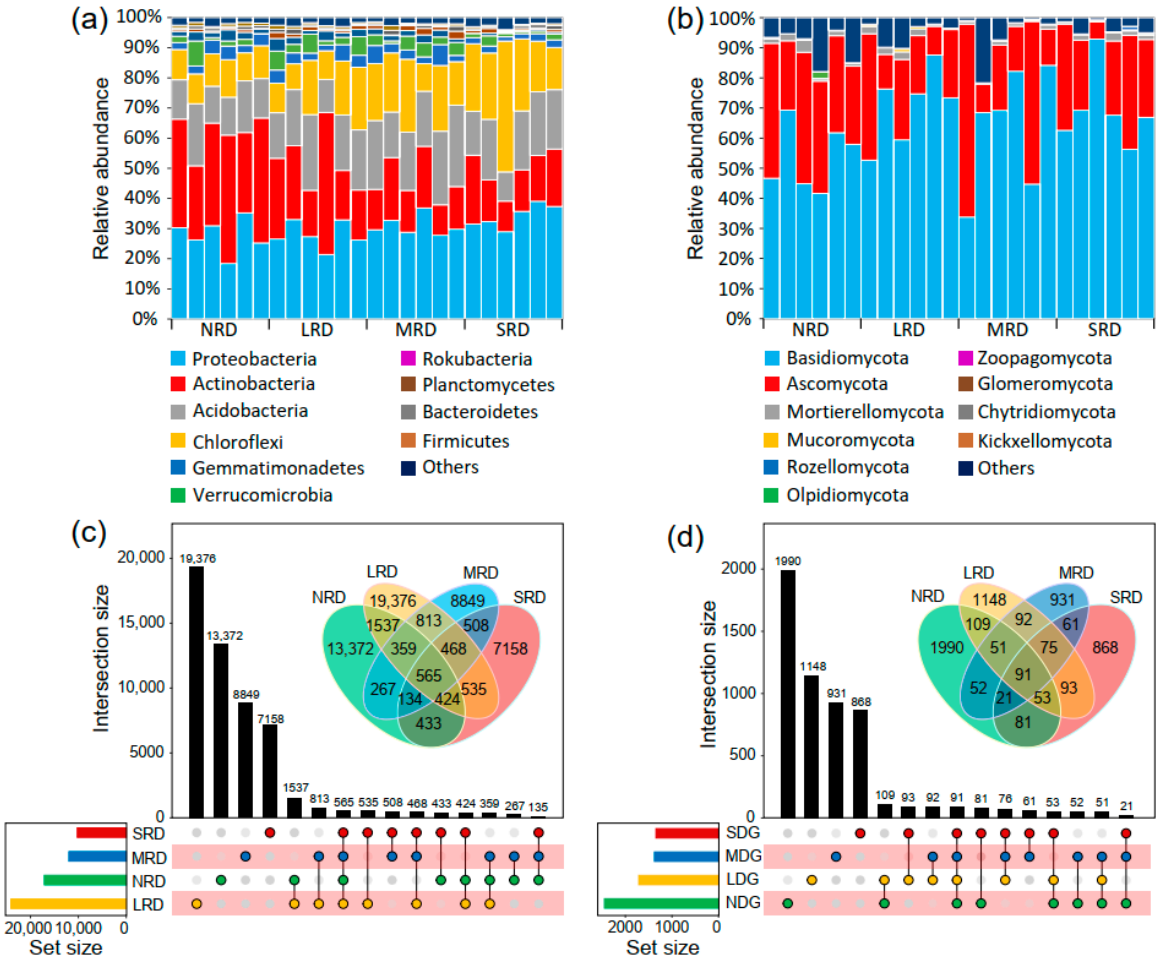

Figure 4. Percentage stacking diagrams show the community composition of bacteria (**a**) and fungi (**b**) at the phylum level. The top 10 phyla were selected for abundance analysis. Venn diagrams show the number of ASVs shared and unique in bacterial (**c**) and fungal (**d**) communities among study sites with four degrees of rocky desertification as shown in a Venn diagram. NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification.

3.4. Composition and β-Diversity of Microbial Communities

To further compare the variations in the structure of bacterial and fungal communities in study sites with different degrees of rocky desertification, based on the Bray–Curtis algorithm, principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) were employed (Figure 5). Study sites were separated from each other, indicating large differences in the structure of microbial communities at different degrees of rocky desertification. In addition, the degree of dispersion of fungal communities between NRD and LRD forests was smaller, suggesting smaller differences in the structure of fungal communities compared with bacterial communities between NRD and LRD forests. Furthermore, microbial β-diversity was calculated using Bray–Curtis dissimilarity based on the ASV table, and the results showed that the β-diversity of bacterial communities decreased with increasing rocky desertification (Figure 5e). The β-diversity of fungal communities in NRD forest was higher than that in SRD forest, while there was no difference between either NRD and LRD forests or MRD and SRD forests (Figure 5f).

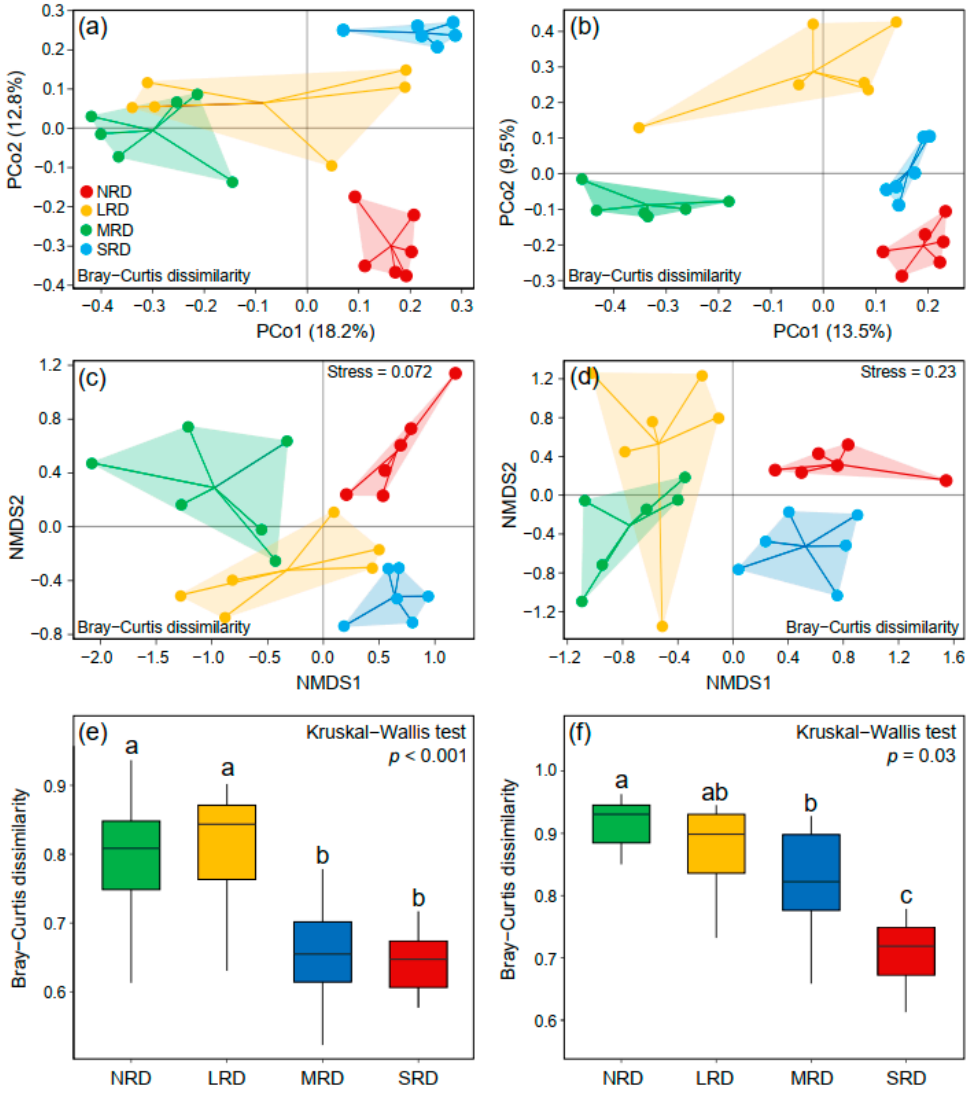

Figure 5. Principal coordinate analysis (PCoA), non-metric multidimensional scaling (NMDS) analysis, and Bray-Curtis dissimilarity of bacterial (**a**,**c**,**e**) and fungal (**b**,**d**,**f**) communities among study sites with four degrees of rocky desertification. Different letters indicate a significant difference at $p < 0.05$ according to Duncan's multiple range test. NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification.

3.5. Indicator Taxa for Different Degrees of RD

Indicator taxa analysis was performed to explore whether bacterial or fungal taxa could be detected as being representative of specific degree of RD based on IndVal analysis (IndVal.g > 0.6, IndVal.p < 0.05), as shown in Figure 6. Six bacterial phyla were identified to be indicator taxa for different degrees of RD: Actinobacteria for NRD forest, Planctomycetes for LRD forest, Dependentiae for MRD forest, and Chloroflexi and Proteobacteria for SRD forest. However, there is no fungal phylum that could be considered as an indicator after calculation. For this reason, we computed the indicator analysis on the fungal order dataset. Finally, twelve fungal orders were selected and could be regarded as indicator taxa for different degrees of RD: Chaetosphaeriales, Diaporthales, Hysteriales, Pleosporales, Trechisporales, and Trichosphaeriales for NRD; Geminibasidiales, GS25, and Umbelopsidales for LRD; Leucosporidiales for MRD; and Atheliales and Cantharellales for SRD.

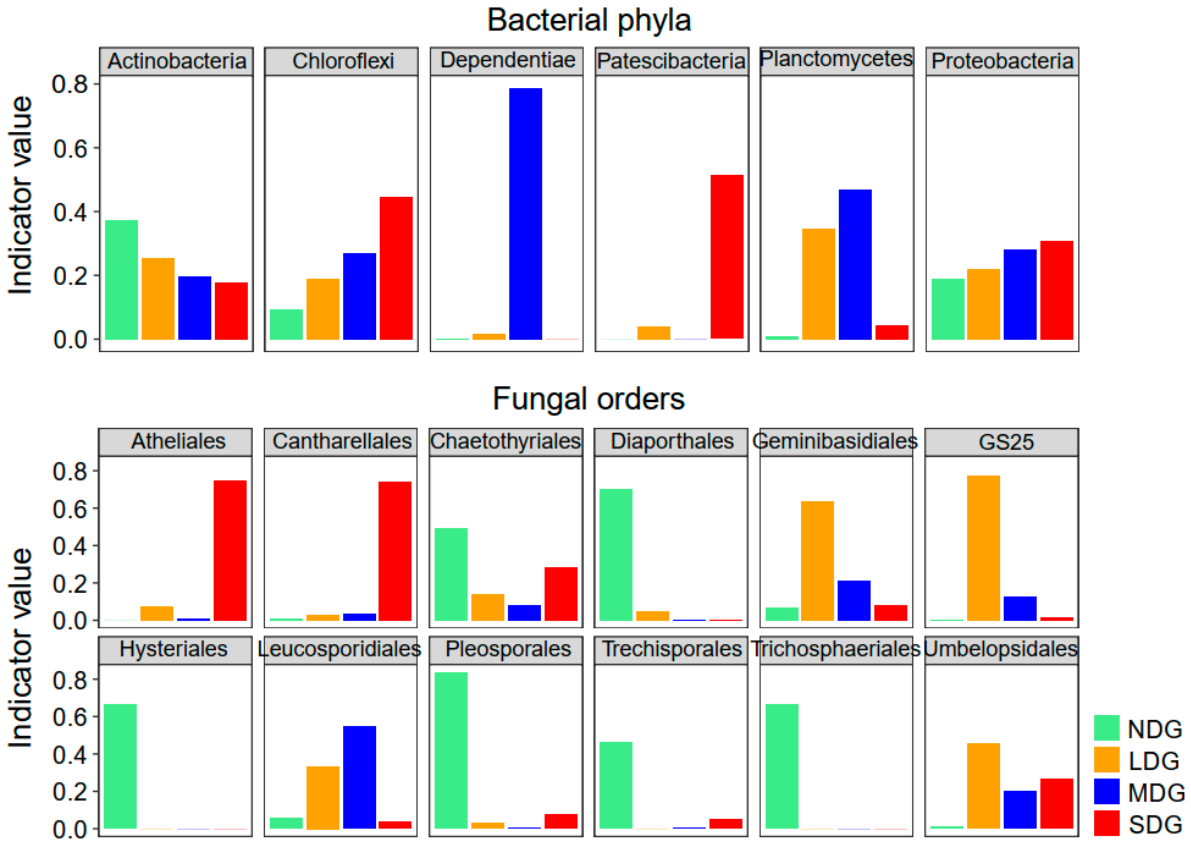

Figure 6. Indicator taxa identified for study sites with different degrees of RD. Values were obtained from the IndVal function, including IndVal.g (>0.6) and IndVal.*p* (<0.05). NRD, non-rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification.

3.6. Main Factors Driving Soil Microbial Communities

As shown in Figure 7, the first and second ordination axes of distance-based redundancy analysis (db-RDA) totally explained 15.07 and 17.84% of the soil bacterial and fungal community changes, respectively. Soil microbes in NRD and LRD forests gathered in the second and third quadrants, which were obviously positively correlated with TP, and also have a certain correlation with SOC, AP, pH, and BD. The microbial communities in MRD forests were mainly distributed in the fourth quadrant, and were mainly restricted by the SOC and AN. The distribution of fungal communities in SRD was relatively scattered, and was mainly affected by pH, TP, and AP.

To explore the correlation between soil microbial community structures and environmental variables, Mantel test analysis was performed with community matrices at OTU level and environmental factors on four gradients of RD (Figure 7c). The results revealed that elevation, BD, pH, SOC, AP, and AN were the determinants of both soil bacterial and fungal community structures among study sites.

Based on the multiple regression model and random forest analysis, the interdependence between the soil microbial community and environmental factors was studied, and the influence of environmental factors on the soil microbial community in different RD forests was further elaborated (Figure 7d). We confirmed that BD, pH, SOC, and AN were the most important environmental factors, showing a strong correlation with microbial community richness, α-diversities, and β-diversities.

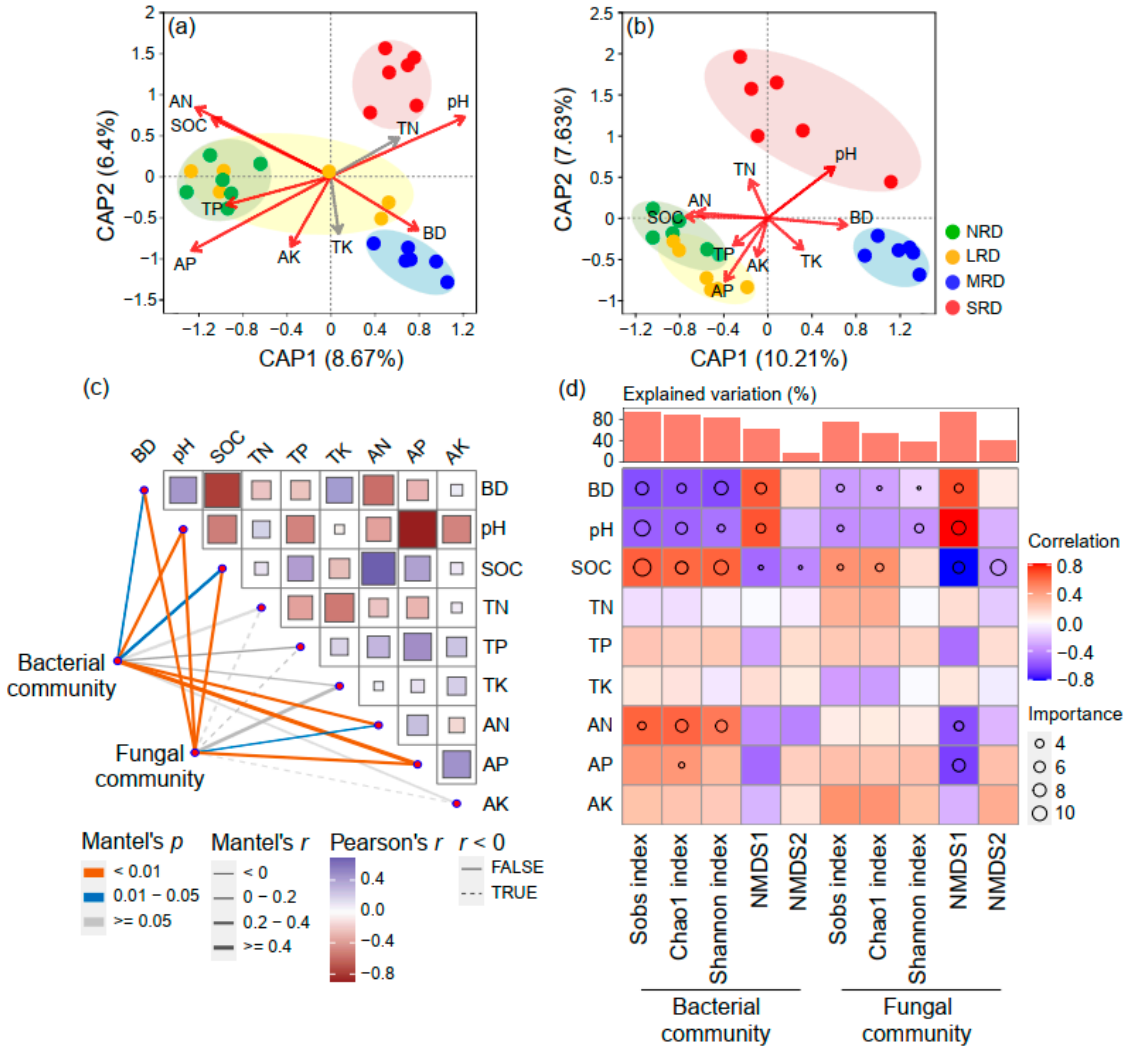

Figure 7. Relationship between soil physical/chemical properties and the diversity and compositions of soil bacterial and fungal communities in forests with different degrees of RD. Distance-based redundancy analysis (db-RDA) ordination plots of bacterial (**a**) and fungal communities (**b**) and soil properties. The environmental variables that significantly explained variability in microbial communities are shown in red color. (**c**) Mantel tests between the microbial communities and soil physical/chemical properties. The upper right corner presents the correlations (Pearson) among the nine soil properties. The lower light corner presents the correlations (Mantel test) between microbial communities (bacterial community and fungal community) and soil properties. Line width represents the significant correlation coefficients of the partial Mantel tests. The line color denotes the significance level. Line formatting indicates the correlation sign (a solid line means a positive correlation, while a dashed line means a negative correlation). (**d**) Correlation between microbial diversity and soil properties, and the main predictors of microbial diversity based on random forest model. The mean predictor importance (% of increase in MSE) indicates the importance of environmental drivers on microbial diversity. NRD, non−rocky desertification; LRD, light rocky desertification; MRD, moderate rocky desertification; and SRD, severe rocky desertification. BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; and AK, available potassium.

4. Discussion

Rocky desertification is a common ecological problem in soil ecosystems worldwide. In this study, we revealed the effects of RD on soil properties, and soil bacterial and fungal communities, in the karst area of southwest China, uncovered the driving factors of the

diversity and compositions of soil bacterial and fungal communities, and explored the indicator taxa for different degrees of RD.

4.1. Effects of RD on Soil Properties

Soil bulk density and porosity are crucial indicators of soil health, directly reflecting the soil's structure and its ability to hold water and resist erosion [29]. The present study showed that RD significantly altered soil physical properties in the karst area of southwest China. The high quality and quantity of plant litters, as well as low human disturbance in NRD forests, promoted more efficient accumulation of soil organic matter, which probably contributed to the relatively low BD in these study areas. With the increase in RD, soil bulk density (BD) increased continuously (Figure 2), which is consistent with other studies from various ecosystems [30,31]. In the alpine meadow of the Tibetan Plateau, grassland degradation significantly increased BD, from 0.99 g cm^{-3} of non-degraded grasslands to 1.38 g cm^{-3} of extremely degraded grasslands [32]. Additionally, in the forest of the Savan watershed, degraded forest (1.29 g cm^{-3}) exhibited higher BD than natural forest (1.03 g cm^{-3}) [33]. The high BD can be attributed to low soil moisture, loss of organic carbon, and weak soil structure. There was a negative correlation between the stock of soil organic carbon and soil bulk density in arid and semi-arid areas of North Africa [34]. In this study, RD caused the reduction in vegetation coverage and productivity, while increasing bare rock area. Subsequently, the soil water evaporation increased and soil water-retention capacity decreased. Additionally, RD exhibited negative effects on soil clay content while leading to an increase in soil sand content [35]. Moreover, wind erosion is one of the most crucial drivers in the RD process of the karst region [36]. Wind erosion breaks down aggregates and wind removes fine particles, mainly clay, increasing the soil's susceptibility to be further degraded [37]. The low vegetation coverage combined with the effect of wind erosion on the karst area destroyed soil structure and accelerated the loss of soil organic carbon. Greater loss of soil organic carbon, lower soil moisture, and poorer aggregation probably account for the higher BD in the RD area in comparison with the non-RD soils (Figure 2).

In addition, RD may affect soil chemistry via direct or indirect pathways, and significantly higher SOC, AN, and AP were found in NRD forests compared with SRD forests (Figure 2). Previous research also found that soil degradation can decrease soil nutrients in several ways. The loss of vegetation coverage caused by RD reduced the protection for soil nutrients, and the reduction in plant productivity decreased the input of soil nutrients from plant litter and root exudates [38]. Soil enzymes are crucial to catalyzing several important reactions necessary for the decomposition of organic matter; thus, their activities play a vital role in nutrient cycling [39]. RD inhibited the activity of soil enzymes, and thus reduced the available nutrients in soils, which explained the lower AN and AP in the SRD forest compared with in the NRD forest (Figure 2). Overall, our results indicated that RD would have adverse effects on soil physical and chemical attributes. Therefore, long-term monitoring of the variations in susceptible indices of soil properties in the early stage of RD helps understand the process of RD and to adjust the management strategies in time [40].

4.2. Effects of RD on the Diversity and Composition of Soil Microbial Communities

Soil microorganisms are a major engine of terrestrial biogeochemistry, playing an essential role in maintaining soil ecosystem functions, including plant growth stimulation, crop residue decomposition, and organic matter turnover [41]. In the current study, we performed high-throughput sequencing of 16S rRNA and ITS1 genes to assess the soil microbial communities of forests with different degrees of RD. RD can alter the diversity and composition of soil microbial communities through various biotic and abiotic factors. The bacterial richness (Sobs index and Chao1 index) and diversity (Shannon index) were lower in the RD forest than in the non-RD forest, which was consistent with a previous study that showed bacterial diversity decreased with the degrees of RD in karst areas [42]. During the RD process, soil nutrients were significantly reduced (Figure 2), which may inhibit

bacterial growth and reproduction [43]. The lost vegetation coverage and productivity in the RD forest was another important reason for the decrease in bacterial and fungal richness [44]. However, our study found that RD did not pose a threat to the soil fungal diversity (Figure 3). This finding agrees with a previous study, which indicated a higher resistance of fungi compared with bacteria [45]. On the one hand, the fungal cell walls are composed of polymers of chitin and melanin, making them quite resistant to degradation. On the other hand, fungi are much more efficient at assimilating and storing nutrients than bacteria, which helps fungi to have a better adaptive ability to poor-resource RD soils. Furthermore, poor-resource RD areas probably provide higher soil heterogeneity and create more niche differentiation for fungal communities than rich-resource non-RD soils [46].

In this study, the bacterial and fungal community compositions in non-RD forests were different from those in RD forests based on both PCoA and NMDS (Figure 5). The dominant phyla in the bacterial microbial communities among different study sites were consistent, including Proteobacteria, Actinobacteria, Acidobacteria Chloroflexi, and Gemmatimonadetes. This result is in line with previous studies performed in the karst region [47,48]. The phylum Proteobacteria is one of the most abundantly distributed bacterial groups in various ecosystems based on numerous 16S rRNA gene surveys from forest, grassland, farmland, and polluted environments worldwide [49–51]. Actinobacteria are considered an indicator of harsh environment, and they play an important role in enhancing the weathering of carbonate rocks [52]. Both 16S rRNA gene-based approaches and shotgun metagenomic assessment have found that Acidobacteria was a highly diverse phylum across a wide range of habitats worldwide [53]. Chloroflexi and Verrucomicrobia are categorized as oligotrophic, having a strong resistance to poor nutrient conditions [54]. In this study, Proteobacteria and Chloroflexi were selected to be indicators for SRD based on IndVal analysis (Figure 6). Nevertheless, the relative abundances of these bacterial phyla were significantly different among study sites. In this study, the relative abundance of Proteobacteria (27.71% in NRD, 27.88% in LRD, 30.90% in MRD, and 34.15% in SRD) and Chloroflexi (10.55% in NRD, 14.06% in LRD, 18.00% in MRD, and 23.72% in SRD) increased with increasing RD, while the relative abundance of Actinobacteria (34.23% in NRD, 24.48% in LRD, 15.49% in MRD, and 15.80% in SRD) reduced gradually (Figure 4). Proteobacteria is one of the largest phyla of soil bacteria, having highly diverse metabolic capabilities, and many members in this phylum can be involved in N_2 fixation and the soil nitrogen cycle [55]. Moreover, Chloroflexi is found in various ecosystems such as soil and water, most of which have a very slow growth rate with an anti-stress strategy in disturbed and nutrient-limited environments [56]. Regarding the fungal communities, Basidiomycota and Ascomycota were the two dominant phyla in this study (Figure 4), and most members in the phyla were involved in carbon cycling through decomposing organic matters [57]. However, the relative abundance of Basidiomycota and Ascomycota presented the opposite trend (Figure 4). This result is consistent with a previous study which indicated that Basidiomycota was higher in degraded soils compared with non-degraded soils [58]. Overall, the results revealed that microbial groups have various adaptive strategies to soil conditions with different degrees of RD in karst habitats [41].

4.3. Factors Shaping Soil Bacterial and Fungal Communities

Extensive studies have indicated that soil variables are the main factors impacting the diversity and composition of soil microbial communities [59]. In the current study, soil pH was one of the most important environmental factors affecting both bacterial and fungal communities in karst areas based on distance-based redundancy analysis (db-RDA), the Mantel test, and the random forest model (Figure 7). Soil pH has been widely accepted as a key factor influencing microbial communities through directly or indirectly influencing microbial growth and soil nutrient availability [60]. Notably, pH impacts the activities of enzymes and each microbial taxa has a definitive pH growth range [61]. Microorganisms grow fast at their optimum growth pH, while growing slowly below the minimum growth pH and above the maximum growth pH. Furthermore, soil pH is regarded as a "master

variable" that has a strong effect on the mobility of compounds in the soil and impacts many biogeochemical processes [62]. Generally, high soil pH can reduce the availability of soil micronutrients, while low soil pH will improve the availability of certain elements such as phosphorus (P), and thereby influence microbial abundance and composition. Moreover, soil pH exerts various effects on bacterial and fungal taxa. For instance, the relative abundances of Chloroflexi and Basidiomycota were positively correlated with pH, while the relative abundances of Actinobacteria, Zoopagomycota, and Kickxellomycota had a negative relationship with soil pH (Figure S1). This result indicated that microbial taxa have their habitat preferences and exhibit different responses to soil pH. It is reported that lower pH decreased bacterial growth and increased fungal growth [63]. On the contrary, bacterial and fungal richness and diversities had the same response patterns to pH in this study: all indices (Sobs index, Chao1 index, and Shannon index) decreased with increasing soil pH. These inconsistent results may be caused by the differences in climate condition, soil and vegetation properties, and the type of degradation among study sites. Furthermore, bacterial and fungal communities were strongly impacted by soil pH, and the bacteria communities (r^2 = 0.947, p = 0.001) were more strongly influenced by pH than the fungi (r^2 = 0.699, p = 0.001), based on db-RDA (Figure 7). This result might be due to the relatively wider optimal pH range for fungal growth than for bacteria growth [64]. The soil BD was also an important factor that impacted the diversity and composition of bacterial and fungal communities in all study sites (Figure 7). Similar results were found in a previous study, which showed that BD had a significant association with the communities of bacteria and fungi in karst areas [65]. It has been accepted that soil BD is an indicator of soil compaction and many key soil processes, such as infiltration, availability of nutrients, and activity of soil microorganisms [66]. High BD with soil compaction leads to the restriction of soil O_2 concentration, and thereby disturbs the microbial diversity and community. It was found that SOC, AN, and AP, but not pH or BD, were the significant variables that explained the majority of the variability in bacterial and fungal diversity and community (Figure 7). By changing microbial activities and plant characteristics, soil nutrients are considered to be an important factor that influences the growth and development of microbes in karst regions [41]. The results were consistent with a previous study that revealed that SOC, AN, AP, and AK were the main factors influencing the microbial community [67]. As a source of nutrients and energy to microorganisms, soil organic matter was reported to play a crucial role in regulating microbial communities [68]. In this study, the relative abundance of Proteobacteria decreased with an increase in SOC and AP, while the relative abundance of Actinobacteria showed a significantly positive correlation with AN and AP. Inconsistent with a previous study [65], there was no correlation between microbial diversity/community and TN and TP in the karst area. This result might be due to the fact that soil microbial taxa vary in their nutrient preferences and nutrient acquisition strategies, which influences the metabolism and species turnover rate of microorganisms. Thus, the current study demonstrated the potential of both soil physical and chemical properties in driving the diversity and composition of bacterial and fungal communities in karst areas, and soil available nutrients (i.e., AN, and AP) might play a more important role in shaping the microbial community than total nutrients (i.e., TN and TP). Notably, further studies are still needed to confirm the effects of environmental factors on shaping diversities and compositions of soil microbial communities in karst areas because the variations in some variables (especially pH and BD) were relatively low in the current study.

4.4. Linkage between Micorbial Indicators and Ecosystem Functions

The remarkable activity of the soil microbiome plays a critical role in numerous vital soil functions, including nutrient cycling, organic matter decomposition, and soil structure formation [19]. Consequently, the positive impacts of combined amendments on enhancing ecosystem health are intricately linked to the diversity and composition of soil microbial communities. In this study, we found that the bacterial phylum Actinobacteria can be considered a good indicator for NRD forests, while Planctomycetes, Dependentiae, Chlo-

roflexi, and Proteobacteria had relatively high abundance in RD forests (Figure 6). Previous research has indicated that Actinobacteria members were clustered to be typical copiotrophic bacterial species, while Planctomycetes and Chloroflexi are typical oligotrophic bacterial species [69]. Here, we detected that the oligotrophic groups (Planctomycetes and Chloroflexi) decreased while copiotrophic groups (Actinobacteria) increased with the RD gradient, suggesting that there was a shift in microorganisms from oligotrophic to copiotrophic groups following forest RD. The oligotrophic taxa with a low growth rate play an essential role in organic matter decomposition [70]. Previous research has revealed that Actinobacteria comprise one of the most abundant and impactful groups of microorganisms within soil microbial communities [71]. They play an essential role in driving ecological nutrient cycling within the soil ecosystem. Notably, these Actinobacteria are renowned for their production of diverse biologically active substances, including enzymes, antibiotics, and vitamins, which contribute significantly to various ecological functions [71]. Furthermore, Dependentiae can be found in most soils with relatively low abundances, although information about their ecological functions remains scant [72]. Unlike the fast-growing copiotrophic taxa, the oligotrophic bacteria prioritize the production of extracellular enzymes during periods of resource scarcity. These enzymes act like molecular scissors, breaking down complex molecules into simpler forms that other organisms can readily utilize.

In addition, Chaetosphaeriales, Diaporthales, Hysteriales, Pleosporales, and Trichosphaeriales belong to the fungal phylum Ascomycota and were found to be enriched in NRD forests (Figure 6). Previous research has indicated that the Ascomycota phylum dominated the fungal communities in harsh environments [73]. These fungi are critical drivers of carbon and nitrogen cycling within these arid ecosystems, playing essential roles in soil stabilization, plant biomass decomposition, and endophytic interactions with plants. By contrast, Geminibasidiales, GS25, Leucosporidiales, Atheliales, and Cantharellales belong to Basidiomycota, and were mainly found in RD forests (Figure 6). Basidiomycota phylum fungi dominate the decomposition of deadwood, with white-rot and brown-rot species playing a pivotal role due to their potent lignin degradation capabilities. These fungal phyla achieve this feat through their impressive arsenal of extracellular lignocellulolytic enzymes, efficiently breaking down the complex lignin polymer [74]. However, further studies are required to reveal the ecological functions of these microbial indicators in forests with different degrees of RD in karst regions.

5. Conclusions

In conclusion, the responses of soil properties and microbial communities to rock desertification (RD) in karst area were systematically investigated. Based on our results, forest RD in this specific area resulted in the variations in the diversities and compositions of soil bacterial and fungal communities. The richness and diversities of microbial communities decreased with increasing degree of RD. The microbial community composition shifted from copiotrophic strategy groups dominating in the non-RD forest, to oligotrophic strategy groups dominating in the RD forest. The variations in soil bacterial and fungal community compositions were mainly explained by soil BD, pH, SOC, AN, and AP. The microbial taxa Chloroflexi, Patescibacteria, Atheliales, and Cantharellales could be regarded as the potential indicators for the specific degree of RD. Taken together, this study aids further understanding of the effects of RD on soil microbial communities and promotes the basic knowledge about the selection of biological indicators for RD forests. However, further studies are required to reveal the relationship between these bio-indicators and ecosystem functions at a large scale to improve activities aiming to restore karst systems via microbial technology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15010047/s1, Method 1: DNA Extraction, pyrosequencing, and bioinformatic processing; Figure S1: The correlations between the relative abundance of microbial phyla and soil properties. BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; and AK, available potassium;

Table S1: Primer sets and thermal profiles used in PCR amplification. References [75–80] are cited in the supplementary materials.

Author Contributions: W.L., Y.Y. and X.W. designed and conceived the experiment. W.L., X.B., S.Z. and Y.C. carried out the experiments and collected the empirical data. W.L., B.H. and Y.Y. performed the data analysis. W.L. wrote the original draft. T.F., Y.Y. and X.W. contributed to revising the paper. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be made available on request.

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References

1. Ford, D.; Williams, P. *Karst Hydrogeology and Geomorphology*; John Wiley & Sons Ltd.: Chichester, UK, 2007.
2. Wang, X.; Lai, J.; Qiu, J.; Xu, W.; Wang, L.; Luo, Y. Geohazards, reflection and challenges in mountain tunnel construction of China: A data collection from 2002 to 2018. *Geomat. Nat. Hazards Risk* **2020**, *11*, 766–785. [CrossRef]
3. Li, Y.B.; Hou, J.J.; Xie, D.T. The recent development of research on karst ecology in Southwest China. *Sci. Geol. Sin.* **2002**, *22*, 365–370.
4. Jiang, Z.; Lian, Y.; Qin, X. Rocky desertification in Southwest China: Impacts, causes, and restoration. *Earth Sci. Rev.* **2014**, *132*, 1–12. [CrossRef]
5. Wang, S.J.; Liu, Q.M.; Zhang, D.F. Karst rocky desertification in southwestern China: Geomorphology, landuse, impact and rehabilitation. *Land Degrad. Dev.* **2004**, *15*, 115–121. [CrossRef]
6. Huang, Q.H.; Cai, Y.L. Spatial pattern of Karst rock desertification in the Middle of Guizhou Province, Southwestern China. *Environ. Geol.* **2007**, *52*, 1325–1330. [CrossRef]
7. Yang, Q.Q.; Wang, K.L.; Zhang, C.; Yue, Y.M.; Tian, R.C.; Fan, F.D. Spatio-temporal evolution of rocky desertification and its driving forces in karst areas of Northwestern Guangxi, China. *Environ. Earth Sci.* **2011**, *64*, 383–393. [CrossRef]
8. Singh, J.S.; Pandey, V.C.; Singh, D.P. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. *Agric. Ecosyst. Environ.* **2011**, *140*, 339–353. [CrossRef]
9. Nikitin, D.A.; Semenov, M.V.; Chernov, T.I.; Ksenofontova, N.A.; Zhelezova, A.D.; Ivanova, E.A.; Khitrov, N.B.; Stepanov, A.L. Microbiological indicators of soil ecological functions: A review. *Eurasian Soil Sci.* **2022**, *55*, 221–234. [CrossRef]
10. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [CrossRef]
11. Delgado-Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Trivedi, P.; Osanai, Y.; Liu, Y.R.; Hamonts, K.; Jeffries, T.C.; Singh, B.K. Carbon content and climate variability drive global soil bacterial diversity patterns. *Ecol. Monogr.* **2016**, *86*, 373–390. [CrossRef]
12. Hernández-Cáceres, D.; Stokes, A.; Angeles-Alvarez, G.; Abadie, J.; Anthelme, F.; Bounous, M.; Freschet, G.T.; Roumet, C.; Weemstra, M.; Merino-Martín, L.; et al. Vegetation creates microenvironments that influence soil microbial activity and functional diversity along an elevation gradient. *Soil Biol. Biochem.* **2022**, *165*, 108485. [CrossRef]
13. Liang, Y.; He, X.; Chen, X.; Su, Y.; Pan, F.; Hu, L. Low frequency of plants associated with symbiotic nitrogen-fixers exhibits high frequency of free-living nitrogen fixing bacteria: A study in karst shrub ecosystems of southwest China. *Forests* **2022**, *13*, 163. [CrossRef]
14. He, Q.; Xiao, Q.; Fan, J.; Zhao, H.; Cao, M.; Zhang, C.; Jiang, Y. The impact of heterotrophic bacteria on recalcitrant dissolved organic carbon formation in a typical karstic river. *Sci. Total Environ.* **2022**, *815*, 152576. [CrossRef] [PubMed]
15. Qi, D.; Wieneke, X.; Tao, J.; Zhou, X.; Desilva, U. Soil pH is the primary factor correlating with soil microbiome in karst rocky desertification regions in the Wushan County, Chongqing, China. *Front. Microbiol.* **2018**, *9*, 1027. [CrossRef] [PubMed]
16. Qi, D.; Wieneke, X.; Xue, P.; He, L.; DeSilva, U. Total nitrogen is the main soil property associated with soil fungal community in karst rocky desertification regions in southwest China. *Sci. Rep.* **2021**, *11*, 10809. [CrossRef] [PubMed]
17. Zhu, H.; He, X.; Wang, K.; Su, Y.; Wu, J. Interactions of vegetation succession, soil bio-chemical properties and microbial communities in a Karst ecosystem. *Eur. J. Soil Biol.* **2012**, *51*, 1–7. [CrossRef]
18. Singh, R.V.; Kaur, J.; Bhagwat, S.; Arora Pandit, M.; Dogra Rawat, C. Deploying microbes as drivers and indicators in ecological restoration. *Restor. Ecol.* **2023**, *31*, e13688. [CrossRef]
19. Delgado-Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Jeffries, T.C.; Gaitan, J.J.; Encinar, D.; Berdugo, M.; Campbell, C.D.; Singh, B.K. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **2016**, *7*, 10541. [CrossRef]
20. LY/T 1840-2009*Technology Regulations of Vegetation Restoration in Karst Desertification Zone*; State Forestry Administration of the People's Republic of China: Beijing, China, 2011.

21. Ma, T.; Deng, X.; Chen, L.; Xiang, W. The soil properties and their effects on plant diversity in different degrees of rocky desertification. *Sci. Total Environ.* **2020**, *736*, 139667. [CrossRef]
22. Bao, S.D. *Soil Agrochemical Analysis*, 3rd ed.; China Agriculture Press: Beijing, China, 2000. (In Chinese)
23. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
24. Sommers, L.; Nelson, D. Determination of total phosphorus in soils: A rapid perchloric acid digestion procedure. *Soil Sci. Soc. Am. J.* **1972**, *36*, 902–904. [CrossRef]
25. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*; USDA Circular 939; U.S. Government Printing Office: Washington, DC, USA, 1954.
26. Keeney, D.R.; Nelson, D.W. Nitrogen inorganic forms. In *Methods of Soil Analysis*, 2nd ed; Agronomy Monograph 9 Part 2; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1982.
27. Isaac, R.A.; Kerber, J.D. Atomic Absorption and Flame Photometry: Techniques and Uses in Soil, Plant, and Water Analysis. In *Instrumental Methods for Analysis of Soils and Plant Tissue*; Walsh, L.M., Ed.; Wiley Online Library: Hoboken, NJ, USA; The Soil Science Society of America: Madison, WI, USA, 1971.
28. Liaw, A.; Wiener, M. Classification and regression by randomForest. *R News* **2002**, *2*, 18–22.
29. Logsdon, S.D.; Karlen, D.L. Bulk density as a soil quality indicator during conversion to no-tillage. *Soil Tillage Res.* **2004**, *78*, 143–149. [CrossRef]
30. Shen, Y.; Yu, Y.; Lucas-Borja, M.E.; Chen, F.; Chen, Q.; Tang, Y. Change of soil K, N and P following forest restoration in rock outcrop rich karst area. *Catena* **2020**, *186*, 104395. [CrossRef]
31. Yang, X.; Zhang, Z.; Guo, X. Impact of soil structure and texture on occurrence of microplastics in agricultural soils of karst areas. *Sci. Total Environ.* **2023**, *902*, 166189. [CrossRef]
32. Zeng, C.; Zhang, F.; Wang, Q.; Chen, Y.; Joswiak, D.R. Impact of alpine meadow degradation on soil hydraulic properties over the Qinghai-Tibetan Plateau. *J. Hydrol.* **2013**, *478*, 148–156. [CrossRef]
33. Davari, M.; Gholami, L.; Nabiollahi, K.; Homaee, M.; Jafari, H.J. Deforestation and cultivation of sparse forest impacts on soil quality (case study: West Iran, Baneh). *Soil Tillage Res.* **2020**, *198*, 104504. [CrossRef]
34. Chenchouni, H.; Neffar, S. Soil organic carbon stock in arid and semi-arid steppe rangelands of North Africa. *Catena* **2022**, *211*, 106004. [CrossRef]
35. Peng, X.; Dai, Q.; Ding, G.; Shi, D.; Li, C. Impact of vegetation restoration on soil properties in near-surface fissures located in karst rocky desertification regions. *Soil Tillage Res.* **2020**, *200*, 104620. [CrossRef]
36. Li, S.; Wei, X.; Huang, J.; Wang, X.; Lu, G.; Li, H. The causes and processes responsible for rocky desertification in karst areas of southern China. Sci. *Cold Arid Reg.* **2009**, *1*, 80–90.
37. Colazo, J.C.; Buschiazzo, D. The impact of agriculture on soil texture due to wind erosion. *Land Degrad. Dev.* **2015**, *26*, 62–70. [CrossRef]
38. Han, R.; Zhang, Q.; Xu, Z. Responses of soil organic carbon cycle to land degradation by isotopically tracing in a typical karst area, southwest China. *PeerJ* **2023**, *11*, e15249. [CrossRef] [PubMed]
39. Zhou, Z.; Gao, T.; Van Zwieten, L.; Zhu, Q.; Yan, T.; Xue, J.; Wu, Y. Soil microbial community structure shifts induced by biochar and biochar-based fertilizer amendment to Karst calcareous soil. *Soil Sci. Soc. Am. J.* **2019**, *83*, 398–408. [CrossRef]
40. Jin, J.; Wang, L.; Müller, K.; Wu, J.; Wang, H.; Zhao, K.; Berninger, F.; Fu, W. A 10-year monitoring of soil properties dynamics and soil fertility evaluation in Chinese hickory plantation regions of southeastern China. *Sci. Rep.* **2021**, *11*, 23531. [CrossRef] [PubMed]
41. Sokol, N.W.; Slessarev, E.; Marschmann, G.L.; Nicolas, A.; Blazewicz, S.J.; Brodie, E.L.; Firestone, M.K.; Foley, M.M.; Hestrin, R.; Hungate, B.A.; et al. Life and death in the soil microbiome: How ecological processes influence biogeochemistry. *Nat. Rev. Microbiol.* **2022**, *20*, 415–430. [CrossRef]
42. Xiao, D.; He, X.; Zhang, W.; Hu, P.; Sun, M.; Wang, K. Comparison of bacterial and fungal diversity and network connectivity in karst and non-karst forests in southwest China. *Sci. Total Environ.* **2022**, *822*, 153179. [CrossRef]
43. Dai, T.; Wen, D.; Bates, C.T.; Wu, L.; Guo, X.; Liu, S.; Su, Y.; Lei, J.; Zhou, J.; Yang, Y. Nutrient supply controls the linkage between species abundance and ecological interactions in marine bacterial communities. *Nat. Commun.* **2022**, *13*, 175. [CrossRef]
44. Li, D.; Liu, J.; Chen, H.; Zheng, L.; Wang, K. Soil microbial community responses to forage grass cultivation in degraded karst soils, Southwest China. *Land Degrad. Dev.* **2018**, *29*, 4262–4270. [CrossRef]
45. Gao, C.; Xu, L.; Montoya, L.; Madera, M.; Hollingsworth, J.; Chen, L.; Purdom, E.; Singan, V.; Vogel, J.; Hutmacher, R.B.; et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. *Nat. Commun.* **2022**, *13*, 3867. [CrossRef]
46. Schlatter, D.C.; Bakker, M.G.; Bradeen, J.M.; Kinkel, L.L. Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology* **2015**, *96*, 134–142. [CrossRef]
47. Li, Q.; Qiu, J.; Liang, Y.; Lan, G. Soil bacterial community changes along elevation gradients in karst graben basin of Yunnan-Kweichow Plateau. *Front. Microbiol.* **2022**, *13*, 1054667. [CrossRef] [PubMed]
48. Lu, Z.X.; Wang, P.; Ou, H.B.; Wei, S.X.; Wu, L.C.; Jiang, Y.; Wang, R.J.; Liu, X.S.; Wang, Z.H.; Chen, L.J.; et al. Effects of different vegetation restoration on soil nutrients, enzyme activities, and microbial communities in degraded karst landscapes in southwest China. *For. Ecol. Manag.* **2022**, *508*, 120002. [CrossRef]

49. Janssen, P.H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* **2006**, *72*, 1719–1728. [CrossRef] [PubMed]
50. Nagarajan, V.; Tsai, H.C.; Chen, J.S.; Hussain, B.; Koner, S.; Hseu, Z.Y.; Hsu, B.M. Comparison of bacterial communities and their functional profiling using 16S rRNA gene sequencing between the inherent serpentine-associated sites, hyper-accumulator, downgradient agricultural farmlands, and distal non-serpentine soils. *J. Hazard. Mater.* **2022**, *431*, 128557. [CrossRef] [PubMed]
51. Nicolas, A.M.; Sieradzki, E.T.; Pett-Ridge, J.; Banfield, J.F.; Taga, M.E.; Firestone, M.K.; Blazewicz, S.J. A subset of viruses thrives following microbial resuscitation during rewetting of a seasonally dry California grassland soil. *Nat. Commun.* **2023**, *14*, 5835. [CrossRef] [PubMed]
52. Borsodi, A.K.; Knáb, M.; Krett, G.; Makk, J.; Márialigeti, K.; Erőss, A.; Mádl-Szőnyi, J. Biofilm bacterial communities inhabiting the cave walls of the Buda Thermal Karst System, Hungary. *Geomicrobiol. J.* **2012**, *29*, 611–627. [CrossRef]
53. Kielak, A.M.; Barreto, C.C.; Kowalchuk, G.A.; Van Veen, J.A.; Kuramae, E.E. The ecology of Acidobacteria: Moving beyond genes and genomes. *Front. Microbiol.* **2016**, *7*, 744. [CrossRef] [PubMed]
54. Yang, L.; Barnard, R.; Kuzyakov, Y.; Tian, J. Bacterial communities drive the resistance of soil multifunctionality to land-use change in karst soils. *Eur. J. Soil Biol.* **2021**, *104*, 103313. [CrossRef]
55. Thajudeen, J.; Yousuf, J.; Veetil, V.P.; Varghese, S.; Singh, A.; Abdulla, M.H. Nitrogen fixing bacterial diversity in a tropical estuarine sediments. *World, J. Microbiol. Biotechnol.* **2017**, *33*, 41. [CrossRef]
56. Davis, K.E.; Sangwan, P.; Janssen, P.H. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony-forming soil bacteria. *Environ. Microbiol.* **2011**, *13*, 798–805. [CrossRef]
57. Luo, X.; Wen, D.; Hou, E.; Zhang, L.; Li, Y.; He, X. Changes in the composition of soil microbial communities and their carbon-cycle genes following the conversion of primary broadleaf forests to plantations and secondary forests. *Land Degrad. Dev.* **2022**, *33*, 974–985. [CrossRef]
58. Peng, X.; Liu, J.; Duan, X.; Yang, H.; Huang, Y. Key Soil Physicochemical Properties Regulating Microbial Community Structure under Vegetation Restoration in a Karst Region of China. *Ecosyst. Health Sust.* **2023**, *9*, 0031. [CrossRef]
59. Viruel, E.; Fontana, C.A.; Puglisi, E.; Nasca, J.A.; Banegas, N.R.; Cocconcelli, P.S. Land-use change affects the diversity and functionality of soil bacterial communities in semi-arid Chaco region, Argentina. *Appl. Soil Ecol.* **2022**, *172*, 104362. [CrossRef]
60. Wang, X.; Ren, Y.; Yu, Z.; Shen, G.; Cheng, H.; Tao, S. Effects of environmental factors on the distribution of microbial communities across soils and lake sediments in the Hoh Xil Nature Reserve of the Qinghai-Tibetan Plateau. *Sci. Total Environ.* **2022**, *838*, 156148. [CrossRef] [PubMed]
61. Rosso, L.; Lobry, J.R.; Bajard, S.; Flandrois, J.P. Convenient Model to Describe the Combined Effects of Temperature and pH on Microbial Growth. *Appl. Environ. Microbiol.* **1995**, *61*, 610–616. [CrossRef] [PubMed]
62. McCauley, A.; Jones, C.; Jacobsen, J. Soil pH and organic matter. *Nutrient Manag. Mod.* **2009**, *8*, 1–12.
63. Rousk, J.; Brookes, P.C.; Bååth, E. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. *FEMS Microbiol. Ecol.* **2011**, *76*, 89–99. [CrossRef]
64. Zeng, Q.; Liu, D.; An, S. Decoupled diversity patterns in microbial geographic distributions on the arid area (the Loess Plateau). *Catena* **2021**, *196*, 104922. [CrossRef]
65. Jiang, C.; Sun, X.; Liu, Y.; Zhu, S.; Wu, K.; Li, H.; Shui, W. Karst tiankeng shapes the differential composition and structure of bacterial and fungal communities in karst land. *Environ. Sci. Pollut. Res.* **2023**, *30*, 32573–32584. [CrossRef]
66. Doran, J.W.; Parkin, T.B. Defining and assessing soil quality. In *Defining Soil Quality for a Sustainable Environment. SSSA Special Publication Number 35*; Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A., Eds.; Soil Science Society of America: Madison, WI, USA, 1994.
67. Zheng, X.; Lin, C.; Guo, B.; Yu, J.; Ding, H.; Peng, S.; Sveen, T.R.; Zhang, Y. Effects of re-vegetation restoration on soil bacterial community structure in degraded land in subtropical China. *Eur. J. Soil Biol.* **2020**, *98*, 103184. [CrossRef]
68. Zheng, Q.; Hu, Y.; Zhang, S.; Noll, L.; Böckle, T.; Dietrich, M.; Herbold, C.W.; Eichorst, S.A.; Woebken, D.; Richter, A.; et al. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biol. Biochem.* **2019**, *136*, 107521. [CrossRef] [PubMed]
69. Liang, C.; Schimel, J.P.; Jastrow, J.D. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* **2017**, *2*, 17105. [CrossRef] [PubMed]
70. Wu, X.; Liu, P.; Wegner, C.E.; Luo, Y.; Xiao, K.Q.; Cui, Z.; Zhang, F.; Liesack, W.; Peng, J. Deciphering microbial mechanisms underlying soil organic carbon storage in a wheat-maize rotation system. *Sci. Total Environ.* **2021**, *788*, 147798. [CrossRef] [PubMed]
71. van Bergeijk, D.A.; Terlouw, B.R.; Medema, M.H.; van Wezel, G.P. Ecology and genomics of Actinobacteria: New concepts for natural product discovery. *Nat. Rev. Microbiol.* **2020**, *18*, 546–558. [CrossRef]
72. Li, H.; Wang, J.; Liu, Q.; Zhou, Z.; Chen, F.; Xiang, D. Effects of consecutive monoculture of sweet potato on soil bacterial community as determined by pyrosequencing. *J. Basic Microbiol.* **2019**, *59*, 181–191. [CrossRef] [PubMed]
73. Challacombe, J.F.; Hesse, C.N.; Bramer, L.M.; McCue, L.A.; Lipton, M.; Purvine, S.; Nicora, C.; Gallegos-Graves, L.V.; Porras-Alfaro, A.; Kuske, C.R. Genomes and secretomes of Ascomycota fungi reveal diverse functions in plant biomass decomposition and pathogenesis. *BMC Genomics* **2019**, *20*, 1–27. [CrossRef]
74. Lundell, T.K.; Mäkelä, M.R.; Hildén, K. Lignin-modifying enzymes in filamentous basidiomycetes-ecological, functional and phylogenetic review. *J. Basic Microbiol.* **2010**, *50*, 5–20. [CrossRef]

75. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10–12. [CrossRef]
76. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef]
77. Katoh, K.; Misawa, K.; Kuma, K.I.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
78. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS ONE* **2010**, *5*, e9490. [CrossRef] [PubMed]
79. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Caporaso, J.G. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [CrossRef] [PubMed]
80. McDonald, D.; Price, M.N.; Goodrich, J.; Nawrocki, E.P.; DeSantis, T.Z.; Probst, A.; Andersen, G.L.; Knight, R.; Hugenholtz, P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* **2012**, *6*, 610–618. [CrossRef] [PubMed]