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Linking Soil Bacterial Communities to Soil Aggregates after Afforestation in a Karst Rocky Desertification Region

Jiacheng Lan *, Mingzhi Huang, Junxian Wang, Shasha Wang, Qixia Long, Xue Qi, Kunqian Yue and Lei Liu

School of Karst Science/State Engineering Technology Institute for Karst Desertification Control, Guizhou Normal University, Guiyang 550001, China
* Correspondence: lanjc2016@163.com

Abstract: Afforestation influences soil aggregates and the soil microenvironment, and it also affects soil bacterial communities. However, the interactions between soil aggregation, soil properties, and the bacterial community that occur following afforestation are still unclear and are rarely studied in karst ecosystems. Soil samples were collected from cropland, for reference, and from natural secondary forests and managed forests in a karst rocky desertification region of Southwest China. Soil aggregates were isolated using the wet-sieving method, and the soil bacterial community composition was determined using high-throughput 16S rRNA sequencing. Afforestation promoted significant macro-aggregation ($p < 0.05$) and increased the soil organic carbon (38%), nitrogen (35.4%), exchangeable Ca (78.6%), and soil water contents (4.1%) but decreased the pH and bulk density. The changes in these soil aggregates and soil properties had marked effects on the abundance and composition of the bacterial community. Variation-partitioning analysis showed that, together, the soil aggregates and soil characteristics explained 23.4% of the variation in the bacterial community, and their interaction formed the largest contribution (14.6%). Overall, our findings suggest that both natural and managed afforestation may shift soil bacterial communities by promoting significant macro-aggregation and altering soil properties.

Keywords: afforestation; soil aggregation; soil characteristics; bacterial community; karst region

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1. Introduction

Bacteria are the most varied and plentiful constituents of soil microbial communities [1], with an estimate of 10^9–10^{10} bacteria and 6000–50,000 species per gram of soil [2]. They are involved in the key processes of the soil ecosystem, including carbon [3,4], nutrient cycling [5], and soil-aggregate formation [6]. Soil aggregates provide diverse microhabitats with a range of physicochemical properties [6] due to their spatially heterogeneous microenvironments, accommodating diverse bacterial communities [6,7]. Soil bacteria are very sensitive to environmental changes. A slight change in the environment could lead to considerable changes in the soil bacteria [8]. Afforestation is an important type of land-use change across the world [3] that can cause aggregate formation [9] and alter the soil properties [10], thus forming distinct soil environments that can shape soil bacterial communities [11]. Understanding the soil environmental factors driving changes in the bacterial community could help us to further understand the effects of afforestation on soil bacterial communities.

Tillage affects soil physical properties, mainly in soil aggregates [12]. For example, conventional tillage can disrupt larger aggregates, causing them to transform into smaller aggregates, in calcareous soils in karst areas [13]. Contrarily, afforestation on farmland promotes the aggregation of smaller aggregate fractions into larger fractions [9,14]. Changes in aggregate formation directly influence the soil water (SW) content, soil porosity, organic carbon, nutrient availability, and oxygen availability, forming distinct soil microenvironments that affect soil bacterial communities [6,15]. Soil bacterial communities were found

to be closely correlated with soil aggregates in incubation and field experiments. An incubation experiment revealed that soil aggregation had a direct impact on the development of soil bacterial communities [16]. A number of field studies also identified significant relationships between soil aggregates and soil bacterial communities [4,6,15]. Different tillage methods resulted in the redistribution of soil aggregates, which contributed 50.1% of the variation in the soil bacterial communities [6]. A significant correlation between soil bacterial communities and soil aggregates after afforestation was also observed in northern China [4]. Many previous studies found that changes in soil characteristics, such as the pH, water, clay, carbon, and N levels, remarkably affected the composition and diversity of the soil microbial community under different types of land use and agriculture management [6,17–20]. These results implied that soil properties may play a significant role in shaping soil bacterial communities. However, these studies did not consider the joint effect of soil aggregates and soil physicochemical properties on soil bacterial communities. A limited number of studies have revealed that soil aggregates can alter soil bacterial communities through their impacts on the soil properties. Zhang et al. [15] found that soil macro-aggregation under conservation tillage reduced the soil porosity and oxygen availability and increased the soil moisture, which, in turn, altered the soil bacterial community composition. Soil aggregates had indirect impacts on the soil bacterial community through the soil organic matter (11.17%) and water (10.20%), as well as the available phosphorus (8.06%) content [6]. For these reasons, soil properties should also be considered when assessing the impacts of soil aggregates on soil bacterial communities. Therefore, we hypothesized that afforestation influences soil bacterial communities via changes in soil aggregates and soil properties.

2. Materials and Methods

2.1. Site Description and Experimental Design

The study sites are located in the Huajiang Karst Gorge demonstration area of Zhenfeng County (25°37′20″–25°40′45″ N, 105°37′24″–105°41′30″ E) in Guizhou Province, Southwest China. The climate is characterized as being a dry, hot subtropical valley climate with a mean annual precipitation of 1100 mm and an annual average temperature of 18.4 °C. The area is a typical Karst Plateau Gorge landform, with an altitude ranging from 500 to 1200 m. The soil is calcareous lime soil and is shallow and unevenly distributed throughout the area [21]. The karst area is approximately 45.4 km^2, of which the four categories of rocky desertification (potential, light, moderate, and severe) account for 38.9%, 34.1%, 14.2%, and 12.8%, respectively.

Since the late 1990s, with the implementation of the "Grain for Green" project and the Karst Rocky Desertification Restoration Project, many croplands have been transformed into natural secondary forests (NFs) and managed forests (MFs, *Zanthoxylum bungeanum* plantation). We selected three sites contaiing NF, MF and CL. The distance between the three sites was approximately 0.5–1.5 km. The NF and MF were converted from adjacent CL 15–20 years ago. The soil types and geographical background were similar between the NF, MF, and CL in each site. The CL has continuously been planted with maize (*Zea mays* L.) for at least 50 years. The sowing time was March, and the harvesting time was August. The application of fertilizer to the maize was irregular from March to May each year. In the MF, *Z. bungeanum* was planted at a density of 1000 plant ha^{-1}. Compound and organic fertilizers were utilized based on the stand age, phenology, and crown. The trees were annually pruned after harvesting the pepper so as to improve the conditions and structure of the ventilation and lighting. The NF was dominated by *Koelreuteria paniculata*, *Mallotus philippensis*, *Toona sinensis*, and *Cipadessa baccifera* (Roth.) Miq.

2.2. Soil Sampling

Three plots per site were established in the CL, NF, and MF. Soil samples were obtained from five random points in each plot at a soil depth of 0–10 cm in November 2018 and then combined as a homogenized sample. A total of 27 samples (three sites × three land-use

types × three plots) were collected. Part of the composite soil sample was undisturbed fresh soil, which was first gently broken along natural failure surfaces and then passed through a 10 mm sieve for the separation of the soil aggregates. Another part of the fresh soil sample was immediately stored and maintained at −80 °C for molecular analysis. The remaining soil was air-dried through 2 mm and 0.25 mm mesh for the determination of the soil physical and chemical properties.

2.3. Soil Physicochemical Analysis

The soil samples passed through a 10 mm sieve were separated by wet sieving through 2, 0.25, and 0.053 mm sieves using the method described by Cambardella and Elliott [22]. These soil samples were fractionated into four classes of size fractions, including large macroaggregate (LMA, >2 mm), small macroaggregate (SMA, 0.25–2 mm), microaggregate (MI, 0.053–0.25 mm), and silt + clay (SC, <0.053 mm). The obtained four aggregate fractions were then dried at 60 °C for 48 h, then weighed. The total aggregates' recovery was determined by the ratio of total aggregate weight to total soil weight. The total aggregates' recovery was 97%–100%. The bulk density (BD), SW, exchangeable Ca contents, and pH were measured following the procedure described by Lu [23]. The soil organic carbon (SOC) and N contents were measured using an elemental analyzer–stable isotope mass spectrometer (Vario ISOPOTE Cube-Isoprime, Elementar, Germany). The carbonates in the soils were excluded with HCl (4 mol L^{-1}) for 24 h before analysis.

2.4. DNA Extraction and Sequencing

Bacterial DNA was extracted from 0.15–0.35 g of thawed soil using a FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The DNA quantity and quality were quantified using a spectrometer (NanoDrop2000, Thermo Fisher Scientific, Wilmington, DE, USA). The V3–V4 region of the bacterial 16S rRNA gene was targeted with the primer pair (338F:5′-ACTCCTACGGGAGGCAGCAG-3′, 806R: 5′-GGACTACHVGGGTWTCTAAT-3′). The protocol for polymerase chain reaction (PCR) amplification has been described previously [24]. In brief, PCR was performed in triplicate at a volume of 20 µL containing 2 × 0.8 µL of 5 µM primer, 16.5 µL of ChamQ SYBR Color qPCR Master Mix (Nanjing Nuovizan Biotechnology Co., Ltd., Naning, China), and 2 µL of template DNA under the following conditions: initial denaturation at 95 °C for 5 min, followed by denaturation at 95 °C for 30 s, annealing at 58 °C for 30 cycles lasting 30 s, and then elongation at 72 °C for 1 min. The PCR amplicons were detected using 2% agarose gels electrophoresis, purified with AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and then quantified using a Quantus™ Fluorometer (Promega Corporation, Madison, WI, USA). Sequencing was performed on the Illumina MiseqPE300 platform (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China). The raw FASTQ files were filtered using Trimmomatic and joined using FLASH. The sequences were clustered using UPARSE version 7.0 software and then divided into operational taxonomic units (OTUs) at 97% similarity. Finally, the raw sequences were deposited in the NCBI Sequence Read Archive with the accession number PRJNA820133.

2.5. Statistical Analysis

The effects of afforestation on the soil physicochemical properties were investigated by one-way ANOVA and Bonferroni's multiple-comparison tests ($p < 0.05$). Regression analyses were applied to determine the relationships between the soil properties and soil aggregates. The one-way ANOVA, Bonferroni's test, and regression analyses were conducted using SPSS 17.0 software. Heatmap analysis was applied to determine the similarity of the dominant bacterial (top 50 in OTU) variations in the different samples. Spearman's rank correlation heatmap analyses of the out-level taxonomic ranks were carried out to investigate the relationship of the top 50 OTUs with the soil aggregates and soil properties. Analysis of similarities (ANOSIM) with the Bray–Curtis dissimilarity was performed to determine whether the difference between groups (two or more groups) was

significantly greater than the difference within groups, which represented the variation in the soil bacterial beta-diversity. Redundancy analyses (RDAs) were used to assess the relationships between the bacterial community and soil properties. Variation-partitioning analysis (VPA) was performed to quantify the relative influences of the soil aggregates and soil physicochemical properties on the soil bacterial community composition. The heatmap, ANOSIM, RDA, and VPA analyses were conducted using R software (version 3.3.1) with the vegan package. A value of $p < 0.05$ was considered significant in all the analyses.

3. Results

3.1. Composition of the Soil Aggregates and Soil Properties

After afforestation, on CL, the amount of LMA increased significantly by 352.7% in NF and 139.4% in MF ($p < 0.05$), respectively, while the SMA, MI, and SC fractions decreased by 34.25%, 81.16%, and 84.96%, respectively, in NF, and by 4.2%, 44.70%, and 59.05%, respectively, in MF (Table 1). The afforestation of NF and MF significantly increased the SOC, N, and exchangeable Ca contents (Table 1, $p < 0.05$) by 25.30%, 24.8%, and 96.96%, respectively, in MF, and by 50.64%, 46.06%, and 60.16%, respectively, in NF. The SOC, N and exchangeable Ca contents showed no significant differences between MF and NF. The SW content of NF was significantly higher and the pH was significantly lower than those of CL and MF, whereas no significant difference was observed between CL and MF (Table 1).

Table 1. ANOVA results showing the effects of afforestation on the soil physicochemical properties.

Land-Use Type	CL	MF	NF
LMA/%	12.40 ± 3.34 c	29.69 ± 4.11 b	56.14 ± 3.06 a
SMA/%	59.59 ± 1.17 a	57.08 ± 3.14 a	39.18 ± 2.77 b
MI/%	12.37 ± 2.40 a	6.84 ± 1.08 ab	2.33 ± 0.38 b
SC/%	15.63 ± 2.31 a	6.40 ± 9.76 b	2.35 ± 0.47 b
SOC/(g kg^{-1})	22.02 ± 0.73 b	27.59 ± 1.56 a	33.17 ± 2.23 a
N/(g kg^{-1})	2.54 ± 0.08 b	3.17 ± 0.19 a	3.71 ± 0.22 a
Exchangeable Ca/(g kg^{-1})	6.25 ± 0.51 b	12.31 ± 1.78 a	10.01 ± 2.21 ab
SW/%	24.88 ± 2.35 b	26.18 ± 1.58 ab	31.85 ± 1.46 a
pH	7.85 ± 0.08 a	7.92 ± 0.17 a	7.32 ± 0.09 b
BD/(g cm^{-3})	1.22 ± 0.03 a	1.19 ± 0.02 a	1.17 ± 0.02 a
Bacterial quantity/(copies g^{-1})	$2.93 \times 10^8 \pm 4.30 \times 10^7$ b	$5.02 \times 10^8 \pm 7.19 \times 10^7$ ab	$5.88 \times 10^8 \pm 5.46 \times 10^7$ a

Significant differences between land-use types are indicated by different letters ("a" and "b" and "c") ($p < 0.05$); no significant difference is indicated by the same letters ($p > 0.05$). Means and standard errors. NF—natural secondary forest; MF—managed forest; CL—cropland; LMA—large macroaggregates; SMA—small macroaggregates; MI—microaggregates; SC—silt + clay fractions; SOC—soil organic carbon; N—nitrogen; SW—soil water; BD—bulk density.

3.2. Soil Bacterial Quantity and Community Compositions

The afforestation soils showed significant increases in the soil bacterial quantity compared with the CL soils (Table 1). A Venn diagram showed that the OTUs varied between the three land-use types. A total of 3794 OTUs were common among the three land-use types, accounting for 72.6%–75.6% of the total OTUs (Figure 1). The numbers of unique OTUs were 466, 335, and 462 in CL, MF, and NF, respectively (Figure 1). The ANOSIM results (Figure 2) showed that the land-use types significantly influenced the soil bacterial communities ($r = 0.427$, $p < 0.01$). The heatmap analysis showed that the composition and abundance of the 50 dominant OTUs differed between the afforestation soils and CL soils (Figure 3). The bacterial OTUs of the NF and MF soils were clustered into a group (Figure 3), and the samples from the CL soils were clustered in a single group. Hence, at the OTU level, the soil bacterial communities in the NF and MF sites were rather different from that of the CL site, in line with the ANOSIM analyses. The dominant OTUs in each land-use type were also different. For instance, the NF site was dominated by OTU4200 and OTU490, the MF site was dominated by OTU4825 and OTU2576, and the CL site was enriched in OTU3910

and OTU1820. The most dominant OTUs belonged to the *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota* phyla.

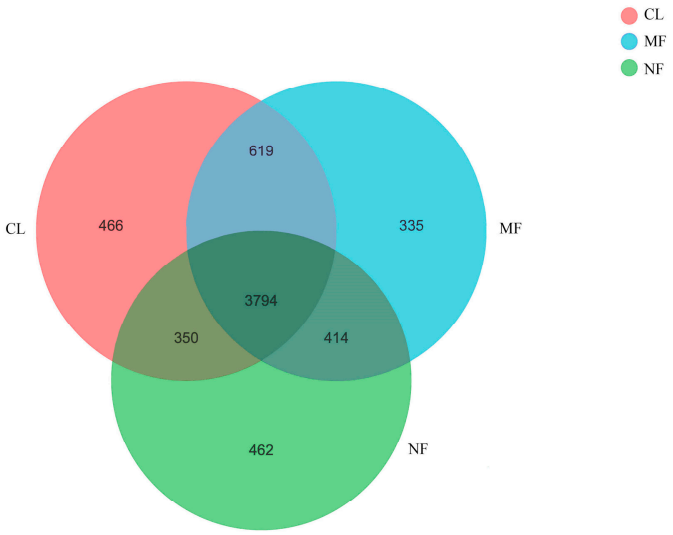

Figure 1. OTU Venn analysis of the soil bacterial communities in the three land-use types. NF—natural secondary forest; MF—managed forest; CL—cropland.

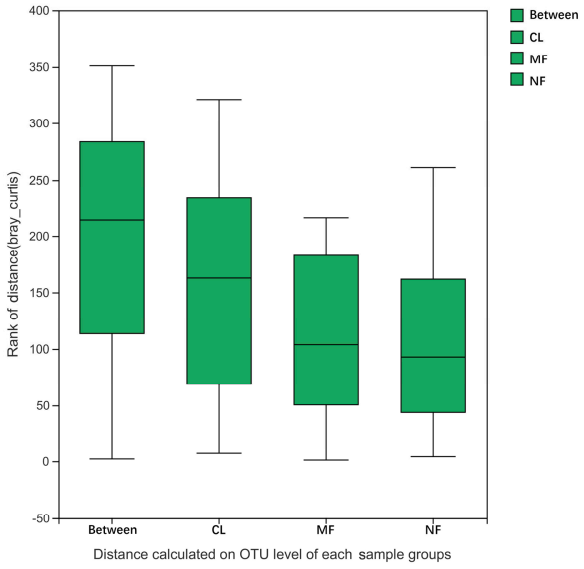

Figure 2. ANOSIM analysis of the distance calculated based on the OTU level for the three land-use types. NF—natural secondary forest; MF—managed forest; CL—cropland.

3.3. Relationships between the Aggregates, and Bacteria and the Soil Properties

Significant positive correlations were observed between LMA and bacterial quantities ($p < 0.05$), and negative correlations were found among MI, SC, and bacterial quantities ($p < 0.05$, Figure 4). The RDA analysis showed that LMA ($r^2 = 0.81$, $p < 0.01$), MI ($r^2 = 0.64$, $p < 0.01$), SMA ($r^2 = 0.60$, $p < 0.01$), and SC ($r^2 = 0.59$, $p < 0.01$) significantly affected the soil bacterial community structure (Figure 5A). The bacterial community structure in NF was more closely associated with LMA ($p < 0.01$), whereas that in CL was more strongly affected by SMA, MI, and SC ($p < 0.01$). The abundance of most of the bacterial OTUs was significantly correlated with the soil aggregates (Figure 6).

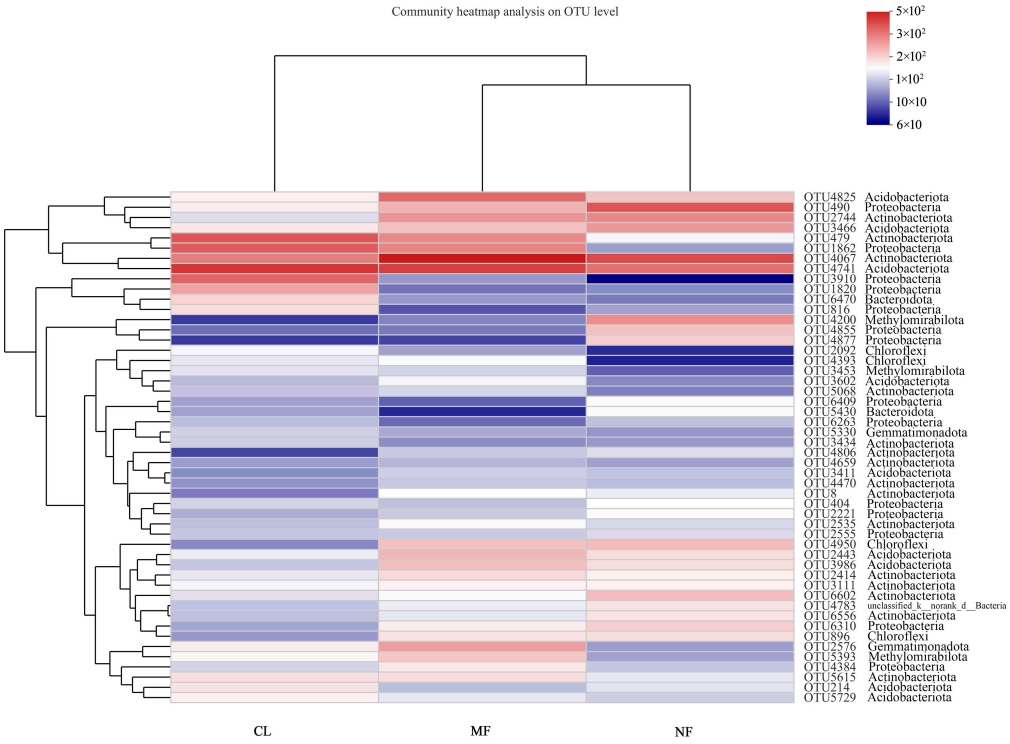

Figure 3. A heatmap diagram of the 50 dominant OTUs in the three land-use types. NF—natural secondary forest; MF—managed forest; CL—cropland.

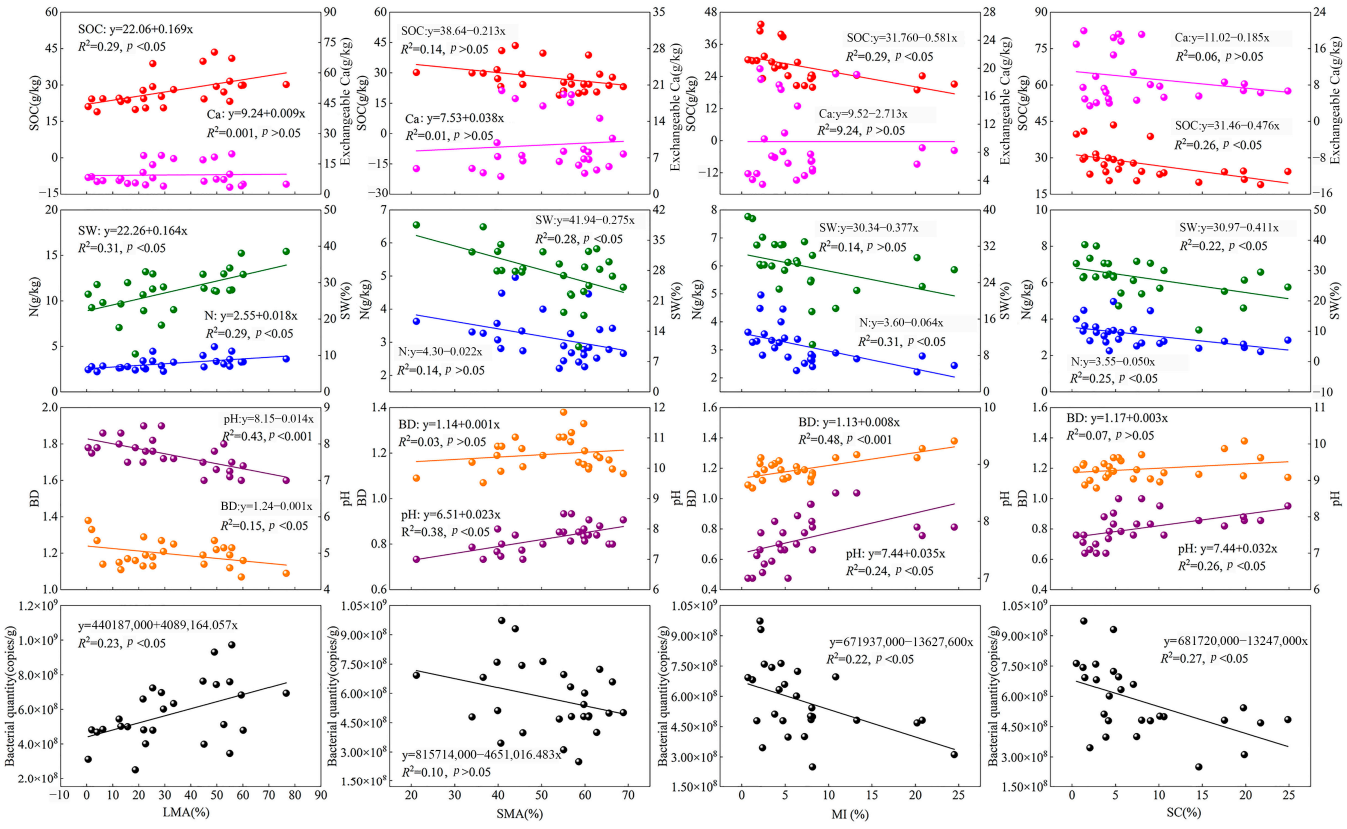

Figure 4. Relationships between the mass proportions of soil aggregates and soil properties. SOC—soil organic carbon; N—nitrogen; SW—soil water; BD—bulk density; LMA—large macroaggregates; SMA—small macroaggregates; MI—microaggregates; SC—silt + clay fractions.

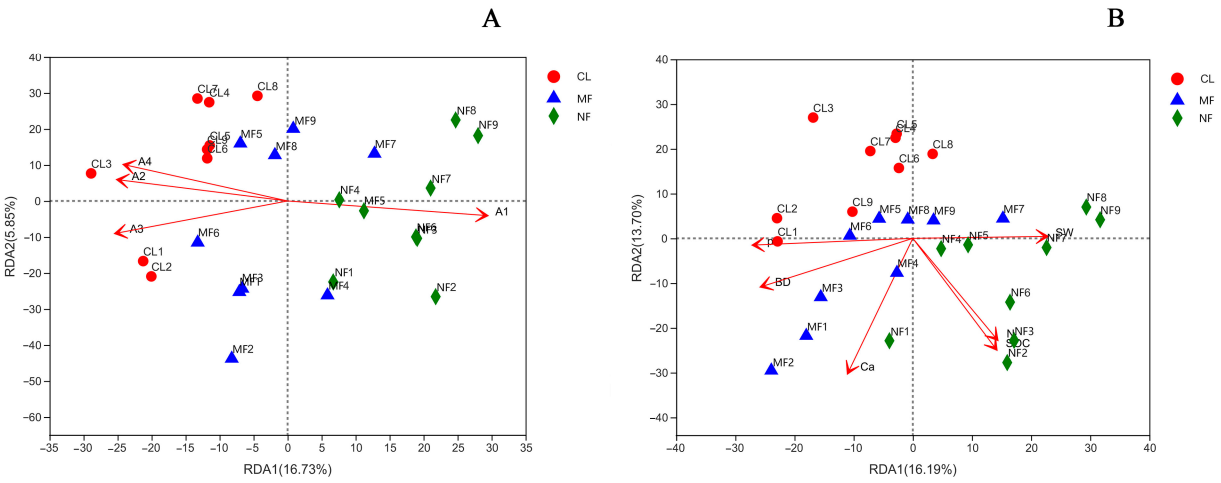

Figure 5. Redundancy analysis (RDA) of the soil bacterial communities with respect to the (**A**) soil aggregates and (**B**) soil properties. NF—natural secondary forest; MF—managed forest; CL—cropland.

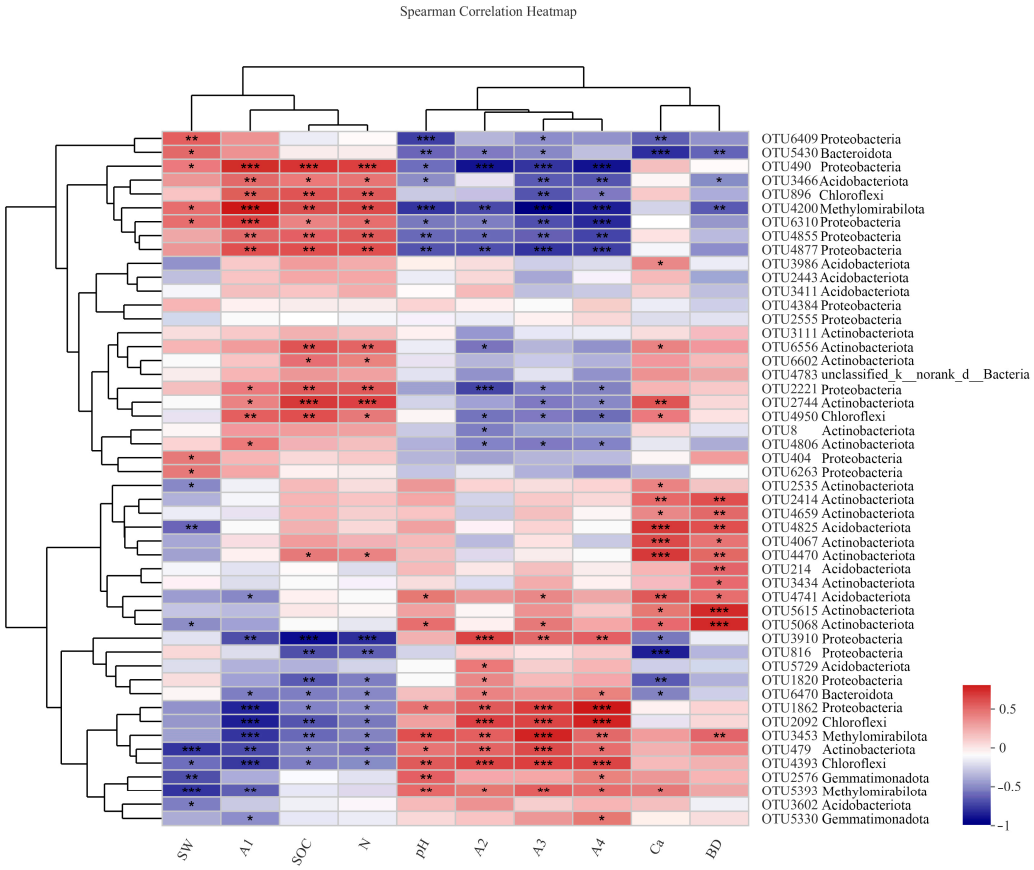

Figure 6. Spearman's correlation cluster heatmap of the soil aggregates and soil properties with respect to the top 50 bacterial OTUs. SOC—soil organic carbon; N—nitrogen; SW—soil water; BD—bulk density; LMA—large macroaggregates; SMA—small macroaggregates;MI—microaggregates; SC—silt + clay fractions. Significance levels are denoted as follows: $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

As shown in the regression analysis in Figure 4, the SOC and N contents were significantly, positively correlated with LMA ($p < 0.05$) but negatively correlated with SMA, MI, and SC ($p < 0.05$). Significant positive relationships were also observed between LMA and SW, whereas a significantly negative relationship was identified between SMA or SC,

and SW ($p < 0.05$, Figure 4). BD showed a negative correlation with LMA, and a positive relationship was identified between BD, SMA, and SC. In addition, pH had a significantly positive relationship with SMA, MI, and SC, and a negative correlation with LMA ($p < 0.05$, Figure 4). No significant relationships were identified between the exchangeable Ca content and the soil aggregates. The exchangeable Ca content showed significant positive correlations with the SOC and N contents (Figure 7).

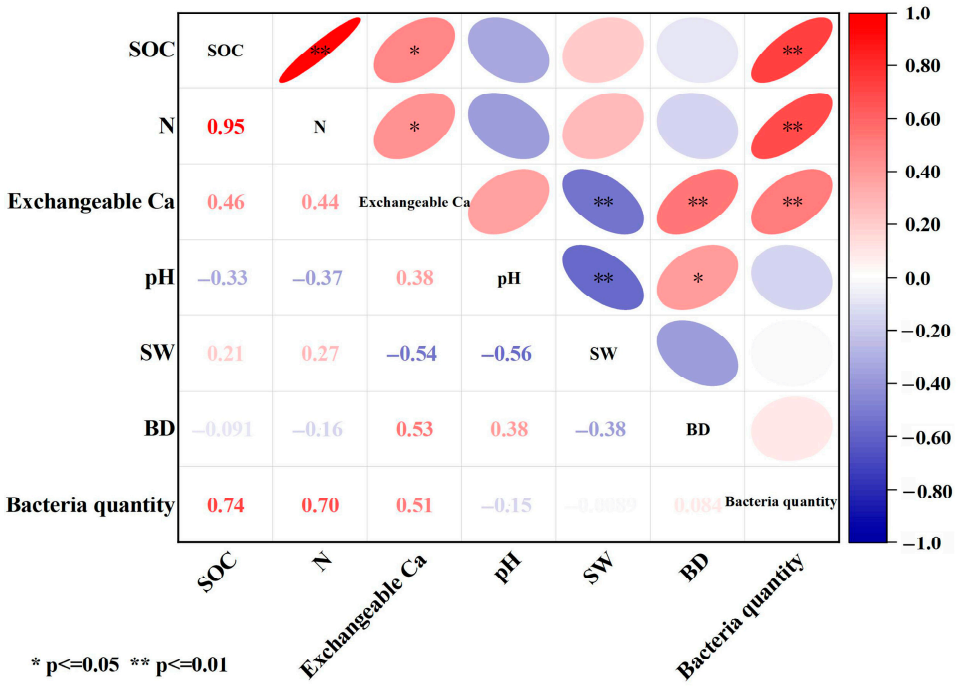

Figure 7. Spearman's correlations among all measured soil properties. SOC—soil organic carbon; N—nitrogen; SW—soil water; BD—bulk density.

3.4. Relationships between the Soil Properties and Quantities, and Bacterial Communities

A significantly positive relationship was identified between the soil bacterial quantities and soil characteristics, including SW, SOC, N, and exchangeable Ca ($p < 0.01$, Figure 7). The RDA revealed that the exchangeable Ca ($p = 0.001$), BD ($p = 0.001$), SOC ($p = 0.001$), pH ($p = 0.001$), N ($p = 0.001$), and SW ($p = 0.003$) had strong effects on the soil bacterial community structure (Figure 5B), with the soil exchangeable Ca being the most significant factor in explaining the total variation. The Spearman's correlation heatmap showed that the abundance of most of the bacterial OTUs was significantly correlated with the soil aggregates (Figure 6).

3.5. Variation-Partitioning Analysis (VPA) of the Soil Bacterial Community, Soil Aggregation, and Soil Properties

VPA further showed that the soil aggregates and soil characteristics jointly accounted for 23.4% of the variation in the bacterial community, while the remainder was left unexplained (Figure 8). The contribution of the soil aggregates only explained 0.5%, while the soil characteristics explained 8.4% of the change in the bacterial community. The soil aggregates and soil characteristics jointly accounted for 14.6% of the variation in the bacterial community, suggesting that a large proportion of the variability in soil properties was associated with the changes in the soil aggregates (Figure 8).

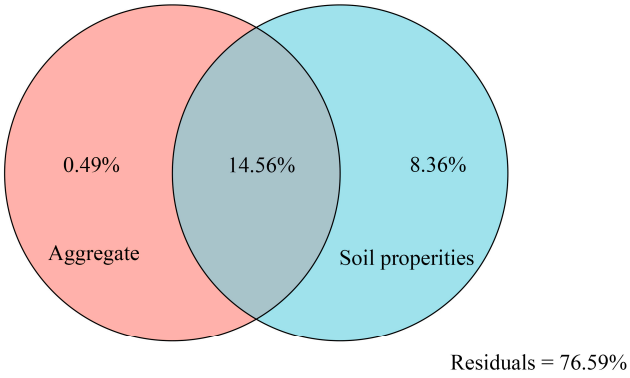

Figure 8. Variation-partitioning analysis (VPA) of the bacterial communities explained by the soil aggregates and soil properties following afforestation.

4. Discussion

4.1. Soil Aggregation and Bacterial Composition Structure

The results showed that both natural and artificial afforestation of former cropland resulted in significant changes in the soil aggregates and the bacterial community. The study also showed that NF and MF demonstrated a significantly increased LMA proportion, whereas SMA, MI, and SC proportions significantly decreased compared with those of CL. Similar results were obtained through other studies in karst areas [25–27]. The influences of afforestation on soil aggregates may be attributed to considerable amounts of biomass derived from litter, roots, and exudates, which favor the integration of smaller aggregates into larger aggregates after afforestation [28,29]. Meanwhile, tillage in cropland accelerates the disintegration of larger aggregates into smaller aggregates.

A significant change in the soil bacterial communities after afforestation was observed in the present study. Moreover, afforestation was found to be a main driving factor in shaping the dominant communities at the OTU level (Figure 3), as was also revealed in previous studies [20]. Most OTUs belonged to the *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota* phyla, possibly because they were the most dominant bacterial phyla in all the soils examined in the previous study [24]. Notably, OTU490, OTU4877, and OTU6310 belonged to the *Rhizobiales* genus, and the increase in *Rhizobiales* is usually related to the increase in root biomass and exudates after afforestation. This result is also confirmed by another study, where vegetation recovery increased the *Rhizobiales* populations in a karst region [30].

The bacterial community composition of the afforestation soils was considerably different from that of the CL soils according to the β-diversity analysis (Figure 2). Habitat heterogeneity may have resulted in the differences in bacterial β-diversity [31], as verified by RDA (Figure 5). In the present study, the soil properties, including exchangeable Ca ($p = 0.001$), BD ($p = 0.001$), SOC ($p = 0.001$), pH ($p = 0.001$), N ($p = 0.001$), and SW ($p = 0.003$), had strong effects on the soil bacterial community, with soil exchangeable Ca being the dominant factor in explaining the total variation (Figure 5B). This finding is consistent with the finding that soil pH, soil moisture, exchangeable Ca, SOC, and N were the main properties shaping the soil bacterial community composition throughout the process of vegetation restoration in karst desertification areas of Southwest China [32]. This result was also confirmed by Li et al. [33], who found that soil exchangeable Ca and pH had considerable influences on the soil properties and soil bacterial community composition in the Karst Graben Basin, in Southwestern China. It is noteworthy that bacterial OTUs in the MF and NF soils cluster together, and both natural and artificial afforestation of former cropland resulted in similar changes in the soil aggregates and soil properties. This supports the inference that managed and natural afforestation can have similar effects in direction but different in magnitude on soil aggregates and soil properties. In detail, both natural and artificial afforestation promoted significant LMA aggregation ($p < 0.05$) and increased the SOC, TN, exchangeable Ca, and SW contents, but decreased pH and BD

(Table 1). These environmental factors had strong effects on the soil bacterial community. In particular, soil exchangeable Ca and LMA were the most significant factors in explaining the total variation in the bacterial community (Figure 5). Therefore, LMA aggregation and exchangeable Ca may be important factors driving similar bacterial OTUs in the MF and NF soils. The magnitude effects of MF and NF on soil aggregates and soil properties were different. Thus, magnitude changes in the bacterial community between NF and MF were different. However, the three land-use types shared a total of 3794 OTUs, accounting for 72.6%–75.6% of the total OTUs (Figure 1). This finding indicated that the land-use history may have a lasting impact on the soil bacterial community composition [18].

Overall, afforestation significantly changes soil aggregation and soil bacteria, permitting us to perform an analysis of the link between soil aggregation and the bacteria.

4.2. Linking Aggregates to Soil Bacteria

Bacterial abundance and communities are closely related to soil aggregation, owing to changes in the soil physiochemical properties [16,34]. Soil aggregates offer habitats characterized by high spatial heterogeneity to bacterial communities [6,7]. In the present study, most OTUs were closely correlated with the soil aggregates (Figure 6). In particular, LMA was positively correlated with OTU490, OTU4877, and OTU6310 from the *Rhizobiales* genus, but the <2 mm aggregate sizes were negatively correlated. LMA also had a significant impact on bacterial quantity. The recovery of plant roots after afforestation provided root exudates and SOC that acted as soil particle-binding agents, thus contributing to the formation and stabilization of the soil aggregates. Meanwhile, *Rhizobiales* also increased due to the recovery of the plant roots. The formed LMA was the most important organic carbon reserve in the karst landscape [9,25,26], providing nutrients for bacterial growth. These findings are consistent with and confirmed by previous studies, which stated that the formation of >0.25 or >1 mm aggregates stimulated bacterial growth [6,15].

Aggregates have been shown to influence soil properties, including SOC, SW, and niche availability [34]. In this study, the distribution and amount of aggregates had significant impacts on SOC, N, SW, pH, and BD. A previous study indicated that the formation of macroaggregates (>0.25 mm) could alter the soil microenvironment, including its SOC, C:N ratio, SW, and soil porosity [15]. These changes in the soil environment, in turn, influenced the bacterial composition and abundance of soil [15]. Our results also suggest that soil properties, including SOC, N, SW, exchangeable Ca, pH, and BD, had significant effects on the soil bacterial community and a close relationship with the abundance of most of the OTUs (Figures 5B and 6). Therefore, the increase in the SOC, N, and SW contents and decrease in soil pH and BD, as well as the formation of LMA, significantly increased the soil bacterial quantity and changed the soil bacterial community composition and abundance. The significant relationships between soil properties and soil aggregates indicated that their interaction may influence soil bacterial composition. The VPA analysis (Figure 8) further showed that, together, the soil aggregates and soil characteristics explained 23.4% of the variation in the bacterial community, and their joint explanation was the largest contributor (14.6%). Wang et al. [6] reported that soil aggregates had an indirect effect on the bacterial community, and this indirect effect was mainly related to the soil organic matter (11.17%), the SW (10.20%), and the available phosphorus. Soil exchangeable Ca was the dominant factor among the soil properties in explaining the total variation in the bacterial community in the present study. However, no significant relationships between the exchangeable Ca content and the soil aggregates were identified. Notably, the soil exchangeable Ca had significant relationships with the SOC, N, and SW contents, as well as with the bacterial quantity (Figure 7). Thus, soil aggregates may have an indirect effect on the soil exchangeable Ca content. These results indicated that there were significant interactions between soil aggregation, the bacterial community, and soil properties following afforestation. Compared with CL, afforestation had strong effects on the soil bacterial community at a depth of 0–10 cm, which may be partially due to changes in the soil properties and soil aggregation. However, the fact that various unknown factors

may also affect bacterial community variation implies that it is necessary to consider other factors, such as soil depth and vegetation, in future studies. Overall, determining the links between the soil aggregates, soil physiochemical properties, and microbial communities could provide new insights into, and enable us to fully understand, the impacts of soil aggregates on soil bacterial communities and aid in the development of an effective method to be implemented in ecological restoration projects.

5. Conclusions

Our results demonstrated that afforestation on cropland, by promoting large macro-aggregation, increasing the SOC, N, exchangeable Ca, and SW contents and decreas-ing pH and BD, which, in turn, shape the soil bacterial abundance and community composition. Bacterial OTUs in the MF and NF soils cluster together supports inferred that artificial and natural afforestation can have similar effects on soil aggregates and soil properties. Furthermore, soil bacterial communities are correlated with the soil properties and soil aggregates. The soil aggregates and soil properties significantly jointly explained 14.6% of varatoions in soil bacterial communities. Overall, determining the links between the soil aggregates, soil physiochemical properties, and microbial communities could provide new insights into, and enable us to fully understand, the impacts of soil aggregates on soil bacterial communities and aid in the development of an effective method to be implemented in ecological restoration projects in karst regions.

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Data Availability Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at NCBI BioProject—PRJNA820133.

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