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The Influence of Land Use Patterns on Soil Bacterial Community Structure in the Karst Graben Basin of Yunnan Province, China

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Abstract: Land use patterns can change the structure of soil bacterial communities. However, there are few studies on the effects of land use patterns coupled with soil depth on soil bacterial communities in the karst graben basin of Yunnan province, China. Consequently, to reveal the structure of the soil bacterial community at different soil depths across land use changes in the graben basins of the Yunnan plateau, the relationship between soil bacterial communities and soil physicochemical properties was investigated for a given area containing woodland, shrubland, and grassland in Yunnan province by using next-generation sequencing technologies coupled with soil physicochemical analysis. Our results indicated that the total phosphorus (TP), available potassium (AK), exchangeable magnesium (E-Mg), and electrical conductivity (EC) in the grassland were significantly higher than those in the woodland and shrubland, yet the total nitrogen (TN) and soil organic carbon (SOC) in the woodland were higher than those in the shrubland and grassland. *Proteobacteria*, *Verrucomicrobia*, and *Acidobacteria* were the dominant bacteria, and their relative abundances were different in the three land use types. SOC, TN, and AK were the most important factors affecting soil bacterial communities. Land use exerts strong effects on the soil bacterial community structure in the soil's surface layer, and the effects of land use attenuation decrease with soil depth. The nutrient content of the soil surface layer was higher than that of the deep layer, which was more suitable for the survival and reproduction of bacteria in the surface layer.

Keywords: karst graben basin; land use pattern; bacterial community; next-generation sequencing

1. Introduction

The karst graben basin in the East Yunnan plateau is a special geomorphological formation due to the rifts of the plateau, which were uplifted at the same time [1,2]. This area is also the main area of the "two barriers and three belts" for China's national ecological security. However, due to deforestation of this area over the past several decades, the karst ecosystem has seriously degenerated, thereby affecting soil quality, soil fertility, and ecological conditions, and resulting in abandoned bare land. To fight against this environmental problem, the "Green for Grain" program, or the Natural Forest Protection Project, was launched by the Chinese government in this region [3–5]. Accordingly, the size of the degenerated areas has shrunk with the revegetation process. However, little is known

about how vegetation restoration types affect the soil bacterial community's structure in the karst graben basin.

Vegetation restoration can affect soil microorganism communities, which can regulate the soil's biogeochemical cycles and affect the stability of the soil's ecosystem [6–8]. Although many studies have discussed soil microbial community structures with land use pattern changes worldwide, there are few studies on the influence of land use patterns on the soil bacterial community's structure in karst graben basins. For example, Suleiman et al. compared the bacterial community in the original forest-covered area and grassland for eight years and found that the main bacterial community composition showed little difference [9]. Song et al. found that the number and composition of the soil microbial population in the karst peak-cluster depression were different in farmland, grassland, scrubland, forest plantation, secondary forest, and primary forest [10]. Ederson et al. found that there were significant differences in the community composition among crops, pastures, and agroforestry, as well as young secondary forest (up to 5 years old), old secondary forest (5 to 30 years old), and primary forest sites [11]. To better explore the influence of land use patterns on the soil bacterial community structure in the Luxi county in the Yunnan karst graben basin, the woodland, shrubland, and grassland in a given karst area were selected. Our objectives were to (i) gain insight into the difference of soil bacterial community structure with land use changes, (ii) inquire into the effects of land use patterns with soil depth, and (iii) identify the key factors in determining soil bacterial communities. Our findings provide a basis for understanding the influence of land use patterns on soil bacterial community structures in the karst graben basin of Yunnan province, China.

2. Materials and Methods

2.1. Study Sites

Luxi county (103°30′–104°03′ E, 24°15′–24°46′ N) is located in the north Honghe Hani and Yi Autonomous Prefecture in Yunnan province in the subtropical monsoon climate zone, which is rainy in summer and dry in winter. The rainy season extends from May to October, and the dry season runs from March to April. The average annual precipitation is 2026.5 mm. The average annual temperature is 16.3 °C. Moreover, the rocky desertification in Luxi country is serious.

2.2. Soil Sample Collection

Sampling occurred between the wet and dry season, January 2018. Three 20 × 20 m plots were established for each land use pattern. The minimum distance between plots was 400 m to avoid pseudoreplication. Soil samples were collected from the surface soil (0–10 cm), named the A layer, and the other samples were taken from the deep layer (10–20 cm), named B layer, with a split tube auger 5 cm in diameter. At each plot, six soil cores were selected in an S-shaped pattern to form one soil sample. A total of 18 soil samples from woodland (WL), shrubland (SL), and grassland (GL) were collected. Soil samples were named according to the soil sampling sites (such as SL) and soil layer (A: 0–10 cm) in that particular order (e.g., SLA). The three land use patterns were continuously managed for 15 years. All soil samples were transported to the laboratory immediately after collection in sterile plastic bags on dry ice and divided into two uniform portions. One portion was stored at −80 °C for DNA analysis, and the other part was air dried for physicochemical analysis.

2.3. Physicochemical Analysis

Soil moisture content (moisture), soil temperature (T), and EC were measured in situ by soil three-parameter tachometers (Delta-T Devices Ltd., Moisture Meter type HH2, UK). Soil organic carbon (SOC) was determined by wet digestion using the H_2SO_4 and $K_2Cr_2O_7$ method [12]. Total phosphorus (TP) was determined using the molybdenum blue colorimetric method following $HClO_4$ digestion [13]. Available potassium (AK) was extracted with ammonium acetate and analyzed using a flame photometer [14]. Total nitrogen (TN) was determined by an element

analyzer [15]. Soil pH was determined by a 1:5 (m:V) soil/water ratio and measured by a corrected desktop pH meter of Maitre-Toledo S470-B Seven Excellence [16]. Exchangeable calcium (E-Ca) and exchangeable magnesium (E-Mg) were determined by ammonium acetate exchange-atomic absorption spectrophotometry [17].

2.4. DNA Extraction

DNA was extracted using the Powersoil DNA Isolation Kit (Mobio Laboratories, Inc., Carlsbad, CA, USA). For next-generation sequencing, the V3–V4 region of 16S rRNA genes was amplified using PCR primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTA AT). The PCR products targeting the V3–V4 region of the 16S rRNA genes were purified by using the TIANquick Maxi Purification Kit (TIANGEN Biotech (Beijing) Co., Ltd., China). Then, 16S rRNA gene sequencing was performed on the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) at the MAGIENE (Guangzhou, China).

2.5. Bioinformatic Analysis and Statistical Analysis

According to the barcode sequence, the sequencing data were divided into different sample data, and the barcode sequence was truncated. After splicing each sample with FLASH 1.2.11 software [18], the Cutadapt 1.9.1 software was employed to truncate the sequence of PCR amplified primers and remove fragments (less than 200 bp) [19]. Using the SILVA SSURef 123 NR database as the reference database, chimeric sequences were removed by the UCHIME 4.2 software [20,21]. Afterwards, the processed sequences were clustered with the UCLUST 1.2.22 software in operational taxonomic units (OTUs) according to sequences with more than a 97% similarity, and the OTUs were classified by the UCLUST 1.2.22 software alignment against the most recent SILVA (123) database [22]. The QIIME 1.9.1 software was used to perform an alpha diversity (Chao1, Simpson, Shannon, and Observed OTUs) and beta diversity test on the OTU table [23].

SPSS 25 software (SPSS Inc., Chicago, IL, USA) for a one-way ANOVA was used to analyze the difference soil physicochemical properties and bacterial community structure and diversity in different land use patterns [24]. The least significant difference method was used for multiple comparisons, and the Pearson correlation coefficient method was used for a correlation analysis. The Origin 8.5 software was used to map the abundance of bacterial communities at the phyla level. The OTUs whose numbers were more than 0.5% of the total OTUs were defined as the dominant OTUs. RStudio 3.0.3 software was used to draw the heat map of the dominant OTUs and perform a distance-based redundancy analysis (db-RDA) between the soil bacteria and soil physicochemical parameters [25]. The similarity of the OTUs among samples was analyzed by using the Bray–Curtis and weighted UniFrac distance algorithm of principal coordinate analysis (PCoA) [26]. The network maps of dominant OTUs and soil physicochemical factors were drawn via the Gephi 0.9.2 software [27].

3. Results

3.1. Soil Physicochemical Parameters with Land Use Changes

It can be seen that the soil's physicochemical properties were different in the woodland, shrubland, and grassland (Table 1). The TP, AK, E-Mg, EC, and T in grassland were higher than those in the woodland and shrubland. The TN and SOC in the woodland were higher than those in the shrubland and grassland. Moreover, the soil physicochemical properties, except for some physicochemical properties in woodland, decreased by increasing soil depth.

Table 1. The soil physicochemical properties in different land use patterns.

Name	Land Use Pattern	TP (g/kg)	TN (g/kg)	SOC (g/kg)	AK (g/kg)	pH	Moisture (%)	T (°C)	EC ($ms.m^{-1}$)	E-Ca (cmol/kg)	E-Mg (cmol/kg)
WLA	woodland	0.828 ± 0.020 ab	5.09 ± 0.24 a	61.00 ± 1.96 a	128.47 ± 5.78 b	6.21 ± 0.11 b	37.47 ± 5.04 a	8.60 ± 0.21 c	68.33 ± 4.10 b	5.14 ± 1.15 a	26.82 ± 0.65 c
SLA	Shrubland	0.734 ± 0.025 b	4.11 ± 0.16 b	47.00 ± 2.5 b	124.03 ± 14.05 b	6.72 ± 0.09 a	42.85 ± 2.56 a	11.60 ± 1.12 b	64.33 ± 2.03 b	6.91 ± 0.09 a	36.18 ± 3.48 b
GLA	Grassland	0.941 ± 0.059 a	2.97 ± 0.14 c	33.00 ± 1.47 c	262.17 ± 44.37 a	6.68 ± 0.08 a	40.00 ± 1.42 a	16.53 ± 0.74 a	84.67 ± 2.03 a	6.87 ± 0.08 a	51.56 ± 2.32 a
WLB	woodland	0.640 ± 0.037 a	3.16 ± 0.49 a	35.79 ± 5.32 a	40.40 ± 2.14 b	6.46 ± 0.10 a	36.53 ± 2.98 a	9.37 ± 0.09 c	70.33 ± 6.64 ab	6.64 ± 0.11 a	29.21 ± 0.28 c
SLB	Shrubland	0.595 ± 0.025 a	2.32 ± 0.12 a	24.53 ± 2.13 ab	38.40 ± 3.98 b	6.66 ± 0.08 a	33.07 ± 1.91 a	10.97 ± 0.33 b	56.00 ± 3.00 b	6.85 ± 0.08 a	34.20 ± 1.02 b
GLB	Grassland	0.762 ± 0.084 a	2.34 ± 0.37 a	20.46 ± 3.71 b	127.27 ± 15.21 a	6.55 ± 0.03 a	32.70 ± 1.16 a	12.77 ± 0.34 a	79.33 ± 5.70 a	6.73 ± 0.03 a	39.82 ± 1.05 a

Note: A = 0–10 cm (A layer), B = 10–20 cm (B layer); data are the means ± standard error (means ± SE); different lowercase letters (a, b and c) in the same column represent a significant difference from the different sample points in the same soil layer ($p < 0.05$). TP: total phosphorous, TN: total nitrogen, SOC: soil organic carbon, AK: available potassium, T: temperature, EC: electrical conductivity, E-Ca: exchangeable calcium, E-Mg: exchangeable magnesium.

3.2. Soil Bacterial Community Structure and Diversity

The dominant phyla were different in the three land use types, as well as in the A and B layers, as seen in Figure 1. There were 13 dominant phyla in the A layer (Figure 1a). *Proteobacteria*, *Verrucomicrobia*, and *Acidobacteria* were the most abundant dominant bacteria. The total proportions of the three dominant bacteria in the A layer were woodland (80.22%), shrubland (80.07%), and grassland (59.52%); the *Verrucomicrobia* in grassland were significantly fewer than those in the woodland and shrubland; *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* in grassland were significantly higher than those in woodland and shrubland (Table S1). In the B layer, the total proportions of the three most abundant dominant bacteria were woodland (78.61%), shrubland (67.14%), and grassland (56.03%). Among the three different land use patterns, the composition and proportions of the other dominant phyla were different but not significant.

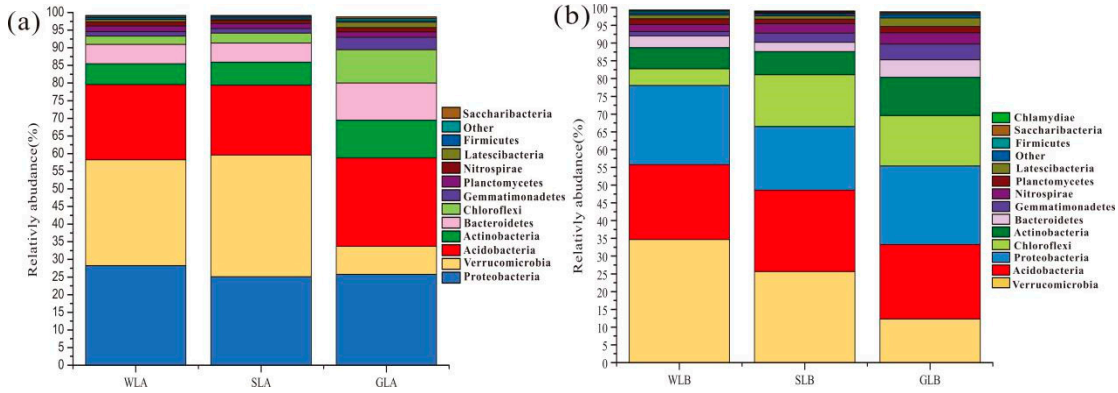

Figure 1. Comparison of the average quantitative contribution of the sequences affiliated with different bacterial phyla from the A layer (**a**) and B layer (**b**). Sequences not classified under any known phylum are included as unassigned bacteria. In each soil sample, the bacterial phylum with relative frequency of less than 1% is included as others.

Moreover, the heat map clearly shows the distribution of the dominant OTUs (Figure 2). In the A layer (Figure 2a), OTU 410 (DA101 soil group) and OTU 24 (*Candidatus Solibacter*) were the dominant OTUs for the woodland; OTUs 238 and 30 (*Candidatus Solibacter*), OTUs 28 and 124 (*Sphingomonas*), OTUs 49, 69, and 27 (subgroup 6), and OTUs 58, 368, 26, and 47 (RB41) were the dominant OTUs for grassland; and OTUs 1, 31, 2312, 9, and 823 (DA101 soil group), and OTU 12 (*Acidobacteria*) were the dominant OTUs for shrubland. In the B layer (Figure 2b), OTUs 163, 823, 2, 1, and 772 (DA101 soil group), and OTUs 10 and 2858 (*Acidobacteriaceae*) were the dominant OTUs in shrubland. OTUs 65 and 16 (0319-6A21) and OTUs 53 and 11 (*Acidobacteriaceae*) were the dominant OTUs in woodland. OTU 21 (*Candidatus Xiphinematobacter*), OTU 71 (*Gemmatimonadaceae*), and OTU 54 (*Pedomicrobium*) were the dominant OTUs in grassland.

The alpha diversities of the soil bacterial communities were different between the three land use types. In the A layer, except for the Simpson index, the alpha diversity indices from grassland were significantly higher than those from shrubland and woodland ($p < 0.05$), as listed in Table 2. Moreover, there was no significant difference between shrubland and woodland ($p > 0.05$). In the B layer, the alpha diversity indices of the three land use patterns were not significantly different, except for the Simpson index. The alpha diversities decreased with an increase in soil depth, except for the Simpson index.

To investigate the effects of land use type and soil depth on soil bacterial communities, we examined the beta diversity (Figure 3). The shrubland and woodland can be accurately clustered together, which shows the similarity of their bacterial communities' compositions. Compared with the A layer, the points in the B layer were more dispersed.

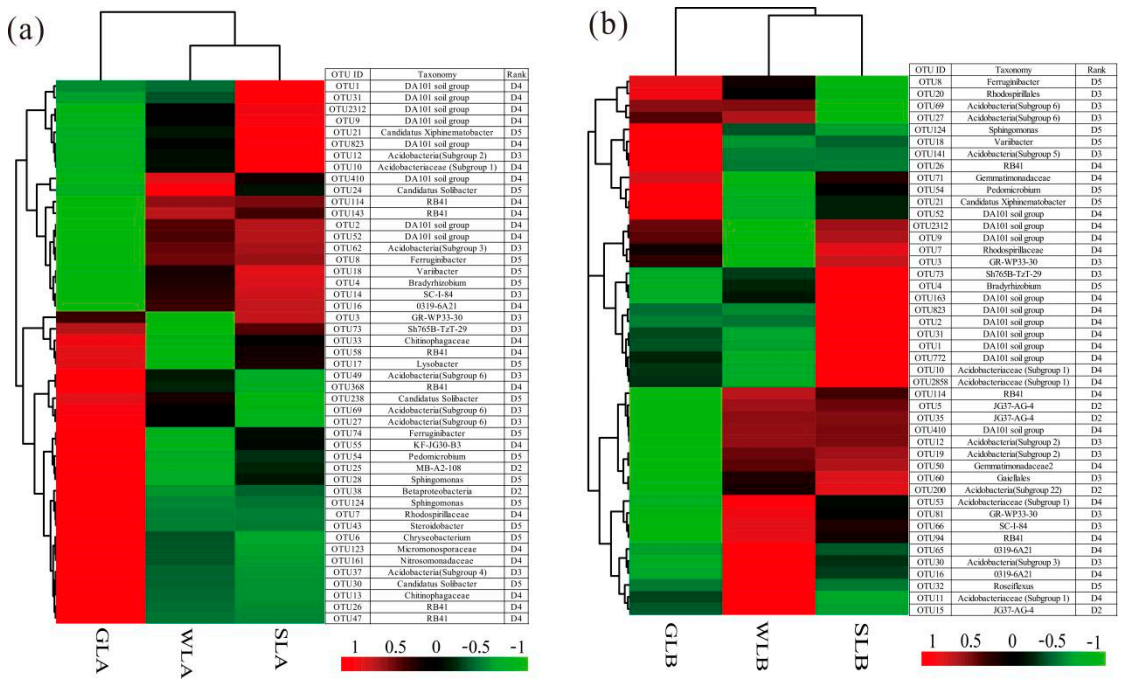

Figure 2. Heat map illustrating the mean relative frequency of the 47 most abundant operational taxonomic units (OTUs) with abundances >0.5% in the A layer with different land uses (**a**). Heat map illustrating the mean relative frequency of the 45 most abundant OTUs with abundances >0.5% in the B layer with different land uses (**b**). Taxonomic assignment of the OTUs is provided at the lowest level of classification possible according to the SILVA 123 database (D1: phylum, D2: class, D3: order, D4: family, and D5: genus).

Table 2. Alpha diversities of the soil bacterial communities with different land use patterns.

Name	Land Use Pattern	Chao1	Shannon	Simpson	Observed OTUs
WLA	Woodland	1656 ± 69 b	8.01 ± 0.23 b	0.985 ± 0.004 a	1162 ± 45 b
SLA	Shrubland	1640 ± 70 b	7.69 ± 0.17 b	0.977 ± 0.004 b	1103 ± 48 b
GLA	Grassland	1935 ± 75 a	8.85 ± 0.10 a	0.993 ± 0.001 a	1409 ± 25 a
WLB	Woodland	1582 ± 89 a	7.80 ± 0.31 a	0.981 ± 0.005 b	1115 ± 67 a
SLB	Shrubland	1565 ± 62 a	7.70 ± 0.07 a	0.982 ± 0.002 a	1030 ± 24 a
GLB	Grassland	1693 ± 119 a	8.54 ± 0.37 a	0.993 ± 0.003 a	1212 ± 106 a

Note: A = 0–10 cm (A layer), B = 10–20 cm (B layer); data are the means ± standard error (means ± SE). Different lowercase letters (a and b) in the same column represent a significant difference from the different sample points in the same soil layer ($p < 0.05$).

3.3. The Relationship between Soil Physicochemical Parameters and Soil Bacteria

To assess the influence of land uses on bacterial community composition, we performed a db-RDA on the dominant bacterial phyla. According to the db-RDA plot and Spearman's correlations between the soil physicochemical parameters and dominant phyla in the land use types, the first two axes accounted for 2.86% of the variability of the bacterial community structure in the A layer. Further, the bacterial communities were positively correlated with six soil physicochemical properties (including SOC, TN, TP, AK, EC, and T) (Figure 4a). *Verrucomicrobia* were negatively correlated with TP, T, E-Mg, AK, and EC, and positively correlated with TN and SOC. *Acidobacteria* were positively correlated with EC, T, and E-Mg; *Actinobacteria* were positively correlated with AK, EC, T, and E-Mg; *Bacteroidetes* were positively correlated with E-Mg, AK, and EC; *Chloroflexi* were positively correlated with T, E-Mg, TP, AK, and EC; and *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* were negatively correlated with TN and SOC (Figure 5). In the B layer, db-RDA showed that the first two axes accounted for 2.86% of the variability of the bacterial community structure, and bacterial communities were positively

correlated with SOC, TN, AK, and T (Figure 4b). *Verrucomicrobia* were positively correlated with SOC and negatively correlated with AK, T, and E-Mg. *Acidobacteria* were negatively correlated with TP; *Chloroflexi* were negatively correlated with TN and SOC, and positively correlated with T and E-Mg; *Actinobacteria* were positively correlated with AK, TP, T, E-Mg, and EC; and *Bacteroidetes* were positively correlated with AK and TP (Figure 5).

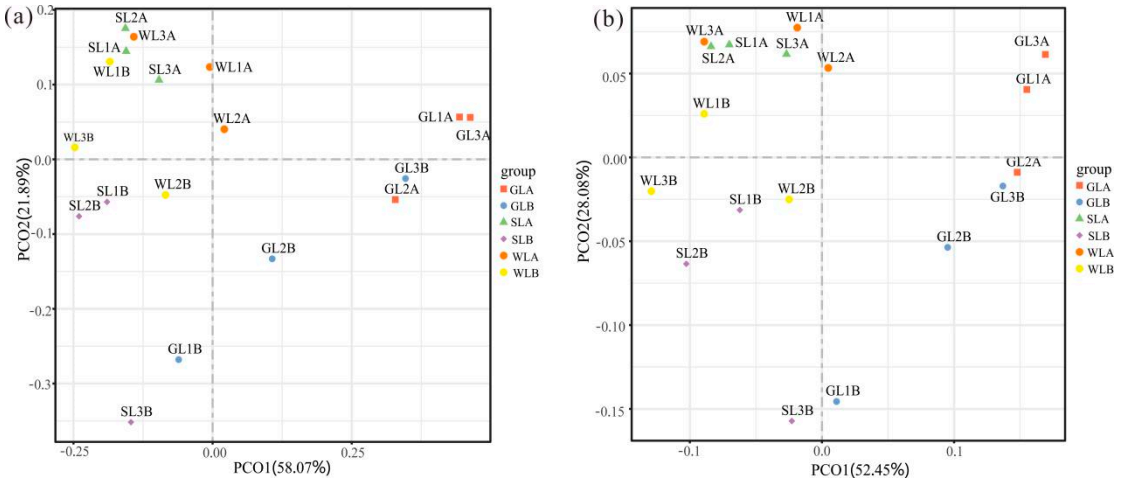

Figure 3. Principal coordinate analysis (PCoA) plots of soil microbial community structures based on the Bray–Curtis and weighted UniFrac results under different land use types in the Yunnan karst graben basin. Bray–Curtis (**a**); weighted UniFrac (**b**).

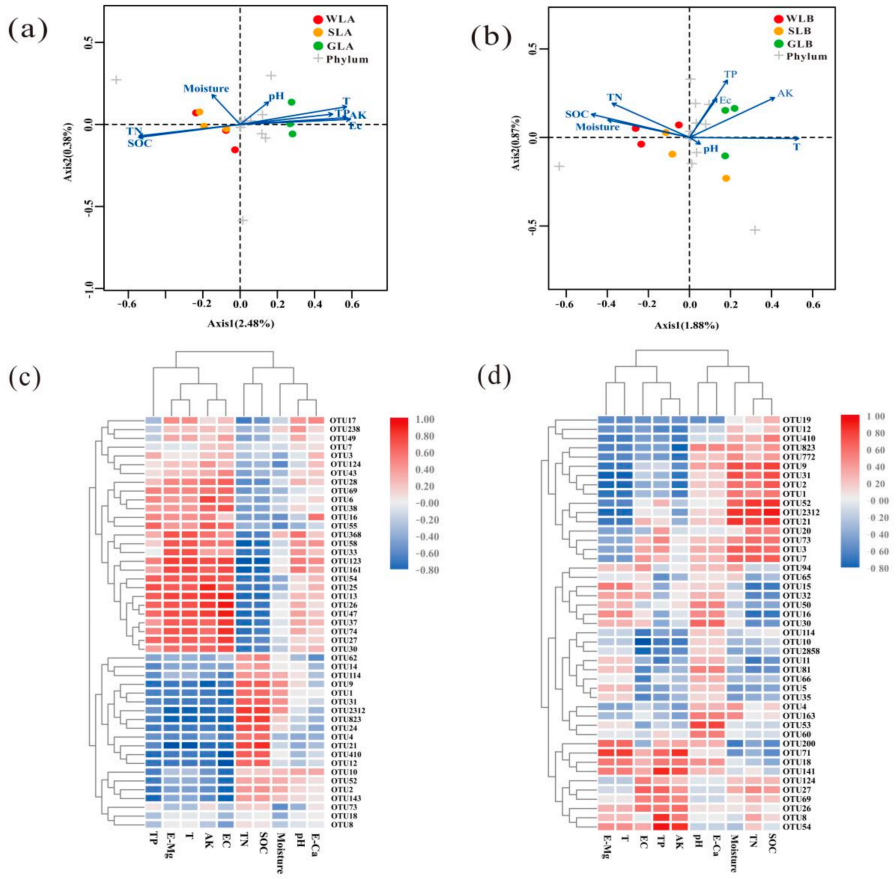

Figure 4. *Cont*.

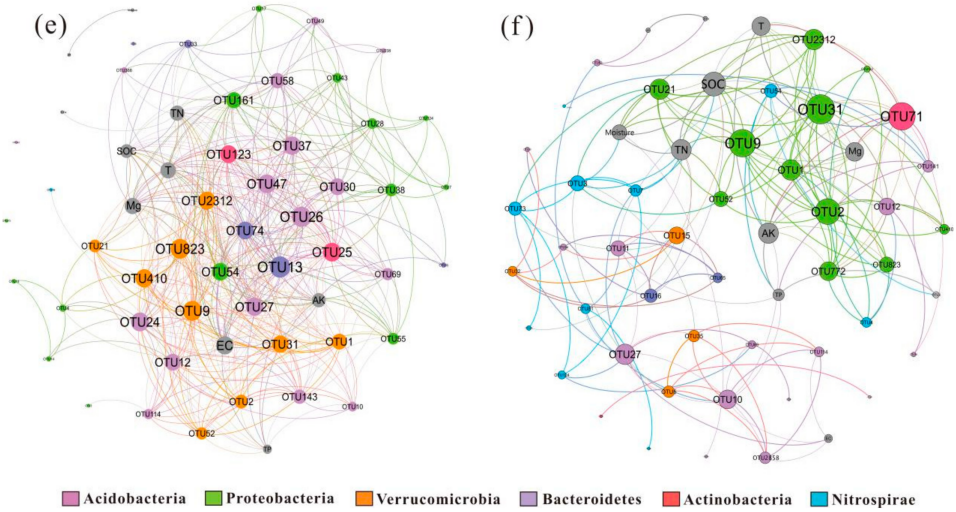

Figure 4. Distance-based redundancy analysis (db-RDA) plots revealing the relationship between the dominant phyla (mean relative frequency >1%) and soil physicochemical parameters in the surface layer (**a**) and deep layer (**b**). Heat map representing the relationship between the soil physicochemical parameters and the most abundant OTUs with abundances >0.5% in the surface layer (**c**) and deep layer (**d**). The networks revealing the co-occurring bacterial OTUs and soil properties in the surface layer (**e**) and deep layer (**f**). The co-occurring networks are colored at the phylum level, and the size of each node is proportional to the number of connections.

A Layer	TP	TN	SOC	AK	pH	T	Moistrue	EC	E-Ca	E-Mg
Proteobacteria	-0.150	0.214	0.283	-0.144	-0.546	-0.395	-0.746*	-0.103	-0.176	-0.395
Verrucomicrobia	-0.691*	0.757*	0.748*	-0.801**	-0.157	-0.775*	0.309	-0.819**	-0.161	-0.775*
Acidobacteria	0.558	-0.611	-0.540	0.565	0.171	0.674*	-0.068	0.777*	-0.089	0.674*
Actinobacteria	0.596	-0.860**	-0.864**	0.820**	0.41	0.830**	-0.132	0.820**	0.327	0.830**
Bacteroidetes	0.666	-0.683*	-0.757*	0.900**	0.114	0.753*	-0.265	0.823**	0.133	0.753*
Chloroflexi	0.739*	-0.776*	-0.816**	0.738*	0.441	0.902**	0.098	0.742*	0.274	0.902**
Planctomycetes	-0.348	0.135	0.254	-0.350	-0.311	-0.180	-0.420	-0.064	-0.439	-0.180
B Layer	**TP**	**TN**	**SOC**	**AK**	**PH**	**T**	**Moisture**	**EC**	**E-Ca**	**E-Mg**
Verrucomicrobia	-0.366	0.581	0.753*	-0.723*	-0.034	-0.845**	0.660	-0.259	-0.028	-0.845**
Acidobacteria	-0.725*	-0.612	-0.423	-0.356	-0.284	0.006	-0.540	-0.568	-0.279	0.006
Proteobacteria	0.472	0.219	0.169	0.354	-0.29	-0.134	0.095	0.503	-0.299	-0.134
Chloroflexi	-0.211	-0.700*	-0.750*	0.193	0.18	0.683*	-0.617	-0.139	0.180	0.683*
Actinobacteria	0.678*	-0.195	-0.406	0.843**	0.239	0.683*	-0.112	0.669*	0.234	0.683*
Bacteroidetes	0.953**	0.437	0.186	0.706	-0.066	0.259	-0.073	0.268	-0.070	0.259
Nitrospirae	-0.386	-0.764*	-0.748*	0.056	0.158	0.534	-0.279	0.143	0.155	0.534
Planctomycetes	0.884**	0.392	0.173	0.641	-0.29	0.153	-0.152	0.251	-0.298	0.153

Figure 5. Spearman's correlations showing the relationship between the soil physicochemical parameters and dominant phyla in the three land use types. Significance levels are denoted as follows: $p < 0.05$ (*) and $p < 0.01$ (**).

Heat maps showed that TP, T, E-Mg, AK, EC, TN, and SOC were significantly correlated with most of the dominant OTUs, whereas pH and E-Ca were significantly correlated with some OTUs (Figure 4c,d). In the A layer, OTUs 26, 47, 58, and 368 (RB41); OTUs 27 and 49 (subgroup 6); and OTUs 30 and 238 (*Candidatus Solibacter*) of *Acidobacteria* were significantly correlated with TP, T, E-Mg, AK, and EC (Figure 4c). Moreover, OTUs 28 and 124 (*Sphingomonas*), OTU 17 (*Lysobacter*), OTU 54 (*Pedomicrobium*), and OTU 43 (*Steroidobacter*) of *Proteobacteria* were also significantly correlated with TP, T, E-Mg, AK, and EC (Figure 4c). In the A layer, OTUs 1, 2, 9, 31, 52, 410, 823, and 2312 (DA101 soil group) and OTUs 114 and 143 (RB41) of *Verrucomicrobia* and *Acidobacteria* were significantly correlated with SOC and TN

(Figure 4c). In the B layer, OTU 26 (RB41) and OTUs 27 and 69 (subgroup 6) of *Acidobacteria* were significantly correlated with TP, T, E-Mg, AK, and EC (Figure 4d), and OTUs 1, 2, 9, 31, 52, 410, 772, 823, and 2312 (DA101 soil group) of *Verrucomicrobia* were significantly correlated with SOC and TN (Figure 4d), as confirmed by their significant relationships in Tables S2 and S3. Although representing the relationship between soil physicochemical parameters and despite the observation that the most abundant OTUs were different in the three land uses, these tables, on the whole, show a certain regularity (Figure 4c,d and Figure S1). The OTU co-occurrence patterns and the relationships among soil bacterial communities and physicochemical parameters were also investigated. Overall, the ecological networks were markedly different among the bacterial groups at different soil depths. This network was comprised of highly connected genera and soil physicochemical properties, thereby forming a "small world" topology. The nodes in the network were assigned to 11 bacterial phyla (Figure 4e,f). Among these, three phyla (*Proteobacteria*, *Acidobacteria*, and *Verrucomicrobia*) were widely distributed, accounting for over 60% of all nodes. In the surface layer, OTUs 26, 47, and 58 (RB41) and OTUs 1, 2, 9, 31, 410, 823, and 2312 (DA101 soil group) of *Acidobacteria* and *Verrucomicrobia* were keystone taxa (Figure 4e). In the deep layer, OTUs 1, 2, 9, and 2312 of *Acidobacteria* were keystone taxa (Figure 4f). This study indicates that these OTUs may play key roles in maintaining the structure and function of soil bacterial communities. It can be seen that EC, E-Mg, SOC, AK, T, and TN were the most important soil physicochemical factors affecting the bacterial community composition in the A layer, whereas SOC, AK, E-Mg, and TN were the most important factors in the B layer. The networks of competition and cooperation were more complex among bacteria in the surface layer than those in the deep layer. The correlation between bacterial communities and soil physicochemical properties decreased with an increase of soil depth (Figure 4c–f).

4. Discussion

4.1. The Characteristics of the Soil Physicochemical Properties

Land use patterns determine the surface vegetation and soil management method, which in turn result in a difference between soil physicochemical properties [28,29]. In our study, TP, AK, E-Mg, EC, and the moisture in grassland were significantly higher than those in the woodland and shrubland. However, the TN and SOC in woodland were significantly higher than those in shrubland and grassland. It is well known that microorganisms are intimately involved in rock weathering and could use these elements as electron acceptors and nutrients [30]. Consequently, grassland, during the early stages of vegetation succession under the influence of microorganisms on rock weathering, had a high concentration of AK, E-Mg, and TP compared with shrubland and woodland. However, due to the deficient root systems and lower amount of litter in grassland, the SOC and TN were low in the grassland. Moreover, SOC and TN are elements of soil fertility and are closely related to ecosystem stability, environmental protection, and land use [31,32]. Compared with grassland, the litter in woodland was high, and the root system was dense. Moreover, TN and SOC were also positively correlated. Therefore, the contents of TN and SOC in woodland were higher than those in shrubland and grassland. Further, soil nutrient contents decreased gradually with increasing soil depth because the soil's surface layer was the humus layer with rich litter, high nutrient content, a well-developed plant root system, good ventilation, and positive hydrothermal conditions [33–36].

4.2. Distribution of Bacterial Diversity Compositions

Bacterial communities from different land use types and different soil depths were highly diverse. In our study, soil bacterial diversity indices from grassland in the A layer and B layer were significantly higher than those in shrubland and woodland ($p < 0.05$). The diversity indices of soil bacterial communities in the three land use types decreased with increasing soil depth, which was consistent with the changes in soil physicochemical properties. Plant community richness usually increases during late successive stages. Moreover, increased plant community richness can significantly alter soil

bacterial community composition and is negatively correlated with bacterial diversity [37]. An increased plant community can supply a diverse array of resources to the soil, thereby promoting coevolutionary niche differentiation and favoring nonantagonistic microbial communities in which antagonism plays little role in maintaining soil community diversity [38]. The decrease in bacterial diversity with increasing soil depth is due to the reduction in the nutrient substrates for bacteria [39]. This is consistent with the expected depth patterns of soil bacterial diversity found in other studies [40,41].

Beta diversity was used to describe the similarities and differences in community structure. The PCoA distance showed a pronounced influence of land use changes on soil bacterial community structure. In our study, bacterial communities from grassland were significantly different from those in woodland and shrubland. This shows that the rate of species replacement between grassland and shrubland was relatively fast, possibly due to the lack of soil nutrients in grassland and increased competition during successive stages, which intensifies the species replacement between communities [42].

4.3. Relationships of Bacterial Communities with Basic Soil Parameters

Soil physicochemical properties determine the living environment of bacteria, which affect soil bacterial communities [43]. Soil bacteria play an extremely important role in organic matter decomposition, soil nutrient cycling, and ecological environment improvement [44,45]. Changes in bacterial community compositions led us to evaluate the extent of different land uses. Then, in our study, 33 dominant phyla were found in the 18 soil samples. *Proteobacteria*, *Verrucomicrobia,* and *Acrobacteria* were the most abundant dominant soil bacteria from the three land use patterns. These phyla have also been found in different relative proportions in other ecosystems worldwide [46,47], suggesting that they may play a fundamental role in these environments.

Proteobacteria showed no significant difference in the three land use patterns, yet their proportion in surface soil (26.61%) was significantly higher than that in deep soil (20.95%), which could be related to their copiotrophic living attributes. *Proteobacteria* belong to aerobic heterotrophic and facultative trophic bacteria [48,49], which are able to live in soils with high organic matter content. As a result, with an increase in soil depth, the soil nutrients decreased, and the abundance of *Proteobacteria* decreased. *Proteobacteria* were not significantly correlated with other physicochemical factors, except for soil moisture (Figure 5). At the same soil depth, the difference in moisture among the three land use patterns was not significant, and the other physicochemical factors had no significant effect on the distribution of *Proteobacteria* because *Proteobacteria* contain many subgroups with different habits, which are distributed in different niches. The *Proteobacteria's* structure is stable, and its anti-interference ability is strong. In the surface layer, OTUs from *Proteobacteria* were more prevalent in grassland (Figure 2a), and most of the OTUs were significantly correlated with TP, AK, E-Mg, EC, and T (Figure 4c). In the deep layer, *Proteobacteria* were not significantly different in their land use (Figure 2b). Further, the OTUs were not significantly correlated with the soil's physicochemical properties (Figure 4d). These bacterial structural differences reflect the prominent changes in particular groups. The heat map revealed that the distribution of OTUs was strongly affected by different land uses and soil depths, and soil nutrients appear to determine the distribution and frequency of OTUs (Figure 4c,d). Among the most frequent *Proteobacterial* OTUs, six were classified at the genus level (*Variibacter*, *Bradyrhizobium*, *Pedomicrobium*, *Steroidobacter*, *Sphingomonas*, *Lysobacter*). Interestingly, some taxonomically related OTUs from land use, such as *Proteobacteria*-related OTUs (28 and 124, classified as part of the *Sphingomonas* group), exhibited similar occurrence patterns. Some recent advances have demonstrated that *Sphingomonas* have unique abilities in degrading refractory contaminants, serving as bacterial antagonists to phytopathogenic fungi [50]. The genus *Bradyrhizobium* (OTU 4) is one of several genera of nitrogen-fixing bacteria, which play an important role in agricultural productivity and global nitrogen cycling. Previous reports have found that *Bradyrhizobium* are dominant in forest soil [51]. However, in our study, *Bradyrhizobium* were found to be dominant in shrubland and woodland environments.

Verrucomicrobia were the most widely distributed and diverse phylum of bacteria in soil habitats [49]. The proportion of *Verrucomicrobia* in grassland was significantly lower than that in woodland and shrubland at different soil depths. *Verrucomicrobia* were distributed in various soil and aquatic habitats, including environments with poor nutrition, eutrophication, extreme pollution, and man-made habitats [52,53]. *Verrucomicrobia* may be more adaptable to oligotrophic environments in soils [54], which are widely distributed in rhizosphere and aggregate soils. They can use a variety of carbon compounds and may be closely related to the carbon cycle [55]. Our study found that *Verrucomicrobia* were significantly positively correlated with TN and SOC (Figure 5), and significantly negatively correlated with TP, AK, EC, and E-Mg. Because the grassland had more soil nutrients, the proportion of *Verrucomicrobia* in grassland was significantly smaller than that in woodland and shrubland. Moreover, it was found that there was a striking increase in verrucomicrobial abundances at different soil depths (Figure 1). The slow growth rate of *Verrucomicrobia* may allow them to exploit the sparse resources in subsurface soil [56]. In different soil layers, OTUs from *Verrucomicrobia* were enriched in the shrubland (Figure 2a,b). Moreover, most OTUs from *Verrucomicrobia* were significantly correlated with TN and SOC (Figure 4c,d). Among the most frequent *Verrucomicrobia* OTUs, only one (OTU 21) was classified at the genus level (*Candidatus Xiphinematobacter*). Previous studies exposed the difficulty of classifying *Verrucomicrobia* because the portion of culturable bacteria within the *Verrucomicrobia* is quite low [57].

Acidobacteria were widespread in all types of soils with high richness [58]. In the A layer, *Acidobacteria* were significantly positively correlated with EC and E-Mg (Figure 5). The proportion of *Acidobacteria* in grassland was significantly higher than that in woodland and shrubland. In the B layer, *Acidobacteria* had a significantly negative correlation with TP (Figure 5), though the proportion of *Acidobacteria* in the three land use patterns was not significantly different. Moreover, the dominant genera from *Acidobacteria* were the *Blastocatella* (OTU 37) and *Candidatus Solibacter* OTUs (21, 30, and 238). In the surface layer, OTUs from *Acidobacteria* were prevalent in the three land uses, reflecting the high adaptability of this group. In the deep layer, OTUs from *Acidobacteria* were more frequent in grassland and shrubland. In addition, most OTUs were significantly correlated with TP, AK, E-Mg, EC, and moisture. Accordingly, each abundant *Acidobacteria* OTU prevailed in a different niche, reflecting the high adaptability of this group. *Acidobacteria* are acidophilic bacteria, which can decompose animal and plant residues and cellulose to form organic carbon [59]. Consequently, they are more suitable for living in a low organic carbon environment. Our study found that *Acidobacteria* were negatively correlated with TOC. Moreover, there was more litter on the soil's surface, making the surface more suitable for the survival of *Acidobacteria*.

5. Conclusions

Proteobacteria, *Verrucomicrobia*, and *Acidobacteria* were the dominant bacteria, but the abundance of bacterial communities was different. *Verrucomicrobia* were significantly positively correlated with TN and SOC and significantly negatively correlated with TP, AK, EC, and E-Mg. *Acidobacteria* were significantly correlated with EC, E-Mg, and TP. Different land use patterns have significant effects on the soil's physicochemical properties. TP, AK, E-Mg, and EC in the grassland were significantly higher than those in the woodland and shrubland. TN and SOC in the woodland were higher than those in the shrubland and grassland. The soil physicochemical properties decreased with increasing soil depth, and SOC, TN, and AK were the most important physicochemical properties affecting the composition of the bacterial community. The diversity of bacterial communities in the three land use types decreased with an increase of soil depth, which was consistent with the trend of soil physicochemical properties. Different land use patterns and soil depths have significant effects on bacterial communities. Land use shapes strong effects in the soil's bacterial community structure on the soil's surface layer, and the effects of land use attenuation decrease with soil depth. Compared with deep soil, surface soil contains more nutrients that are more suitable for the growth and reproduction of bacteria. Our study provides a basis for understanding the influence of land use patterns and soil

depths on the bacterial community structure in the karst graben basin of Yunnan province, China. Selecting a suitable land use according to the soil bacterial community structure of karst graben basins has great significance. We should also consider the impacts of soil bacterial communities on land use at different soil depths.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/1/51/s1: Table S1: Abundance of dominant phyla in the three land use types. Table S2: Spearman's correlations showing the relationship between soil physicochemical parameters and dominant OTUs in the A layer. Table S3: Spearman's correlations showing the relationship between the soil physicochemical parameters and dominant OTUs in the B layer. Figure S1: Heat map representing the relationship between the soil physicochemical parameters and the most abundant OTUs with abundances >0.5% of the three land uses in different soil layers: woodland in the surface layer (**a**), shrubland in the surface layer (**b**), grassland in the surface layer (**c**), woodland in the deep layer (**d**), shrubland in the deep layer (**e**), and grassland in the deep layer (**f**).

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