The Response of Soil Bacterial Communities to Land-use Types in a Subtropical Mountainous Region, Southwestern China

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Abstract: In this study, the characteristics of soil bacterial composition and diversity were investigated under four land-use types (afforestation land, FL; abandoned land, SL; native grassland, GL; cultivated land, CL) to explore correlations between land-use types and the bacterial communities in soil in a subtropical mountainous region of southwestern China. The results showed that the dominant taxonomic genera were Actinobacteria and Proteobacteria. Land-use type influenced the bacterial richness and diversity, and ranked as follows: FL>CL>SL>GL. Marked differences in the distribution of taxon assemblages among the different land-use types in soil were found. The relative abundances of Acidobacteria in SL, Proteobacteria in FL, and Chloroflexi in GL presented as being higher than in other land-use types. Among all the physicochemical properties, significant Pearson linear correlations between soil bacterial taxonomic genera (Flavobacterium, Sphingomonas, Blastocatella, Zymomonas, Pedomicrobium, Reyranella, Rhodanomonas, Flavisolibacter, Candidatus and Telmatobacter) and physicochemical properties (pH, TP and LAP) were observed. These findings suggested that land-use type is a major factor that affects the bacterial composition and diversity, and that changes in soil bacterial composition and diversity are associated with shifts in physicochemical properties.

1. Introduction
For a long time, Guizhou Plateau is one of the most serious regions of poverty and environmental degradation in southwestern China, and it is also overpopulated, and the social economy is lagged[1]. Since the end of 1990s, most of the seriously degraded lands have been subject to ecological restoration due to the implementation of several ecological restoration projects, such as the Green for Grain program and mountain closures (providing living allowances and banning logging)[2-3]. Therefore, land-use types have changed considerably due to changes in the social-economic status of the human population and associated changes in land-use needs. Nowadays, this region has been managed as forestland, shrubland, grassland, and cropland, and about 80% of the original area has experienced land-use change. These changes cause progressive and cumulative soil disturbances that shift intrinsic soil characteristics, by promoting qualitative and quantitative changes in organic matter, carbon and nitrogen, shifting soil moisture, pH, as well as the dwelling microbial communities in this systems[4-6].

Microorganisms (especially bacteria) play unique roles in ecosystem functions, which contribute to soil productivity through their involvement in organic matter decomposition, humus formation, nutrient transformation, and element cycling[7-8]. Many studies have shown that soil microorganisms...
are sensitive indicators of land-use conversion impacts, because they could better reflect changes in soil quality and sustainability\[7,9\]. Despite the importance of soil microbes as a major factor of maintaining ecosystems stability, we know little about the relations between soil bacterial community and land use change in Guizhou plateau, southwestern China. In this study, we researched that the soil bacterial communities respond to land use change, and these responses are related to soil physicochemical alterations caused by different management in the process of land use conversion.

2. Materials and methods

2.1. Study area

The study area (26°19'-26°23'N, 106°50'-106°54'E) was located in Longli county, Guizhou Province, southwestern China. This area has a subtropical humid monsoon climate, and the average annual temperature is 14.8 °C, ranging from -3 °C in January to 35 °C in July. The mean annual rainfall is 1160 mm, more than 85 % rain falls from April to October. The soil type is mainly yellow soil with pH ranging from 4.0 to 6.5. The main types of land-use are sub-alpine meadow, secondary forest and farmland. Based on the types of land use in the study area, we studied four typical land-use treatments: i) afforestation land (FL); ii) abandoned land (SL); iii) native grassland (GL); and iv) cultivated land (CL).

2.2. Soil sampling

In October of 2017, three sampling sites were selected for each land-use type in the study area, using three plots as three independent replicates. The four treatments are located in the same soil type and are close to each other. The plots measured 30 m x 30 m, and the distance between each plot was at least 10 m. The vegetation composition of the three plots at each site, all in well-drained areas, was similar. For the sampling, PVC tubes (5cm diameter by 25cm long) previously sterilized were used to collect the 0-10cm topsoil layer in each plot along an S-shape using a specific coring device with a diameter of 5cm. We stored the samples at 4°C in a portable refrigerator during field sampling. Once in the laboratory, samples were also stored at 4°C until analyses. The fresh soil samples were sieved through a 2-mm mesh and were subdivided into two subsamples. One subsample was stored at −20°C were used for molecular analysis. The other was kept at 4°C until analysis for physicochemical properties of the soil.

Soil moisture (SWC), soil bulk density (BD), pH, SOC, STN, STP, and soil enzyme (β-1, 4-glucosidase, BG; β-1,4-N-acetylglucosaminidase, NAG; leucine aminopeptidase, LAP; acid phosphatase, AP) were measured\[10\]. Information about the soil physicochemical properties is shown in Table 1.

| Soil parameters | Land-use types |
|----------------|---------------|
|                 | FL            | SL            | GL            | CL            |
| pH              | 6.42±0.315a   | 4.075±0.081c  | 4.642±0.035bc | 5.005±0.168b  |
| SWC(%)          | 0.182±0.017c  | 0.158±0.011c  | 0.227±0.007b  | 0.318±0.011a  |
| BD (g cm⁻³)     | 1.169±0.076bc | 1.365±0.049a  | 1.314±0.037ab | 1.029±0.056c  |
| SOC(g.kg⁻¹)     | 17.169±3.346a | 11.247±3.712a | 15.700±0.707a | 15.618±1.158a |
| TN(g.kg⁻¹)      | 10.888±1.591a | 5.033±1.592b  | 10.515±0.704b | 11.484±0.881a |
| TP(g.kg⁻¹)      | 0.273±0.007b  | 0.130±0.009d  | 0.220±0.010c  | 0.346±0.028a  |
| BG (μmol activity g⁻¹ dry soil d⁻¹) | 115.016±17.288a | 32.430±7.866b | 62.475±0.685b | 57.750±13.756b |
| NAG (μmol activity g⁻¹ dry soil d⁻¹) | 23.832±1.14b | 30.233±1.021b | 29.237±4.748b | 58.248±6.352a |
| LAP (μmol activity g⁻¹ dry soil d⁻¹) | 22.910±0.823a | 3.847±1.096c | 2.512±0.519c | 15.412±3.284b |
| AP (μmol activity g⁻¹ dry soil d⁻¹) | 26.685±0.293a | 23.445±1.165b | 26.059±0.160a | 25.982±0.177a |

Note: Letters a, b, c represent significant differences of individual parameters between the four land use types (Duncan test, P< 0.05). Mean ± SEM (n=3)
The structure and composition of soil bacterial communities were measured with MiSeq sequencing of the bacterial ITS region[10].

2.3 Statistical analyses
The significant effects among land-use types were determined and compared by one-way ANOVA, Duncan’s test, and S-K-N multiple range comparison in the SPSS 18.0 statistical software package (SPSS Inc., USA). Pearson’s correlation analyses were used to examine the relationships between the relative abundances of the abundant bacterial genera and physicochemical properties.

3. Results

3.1 Sequencing results and OTU analysis
The MiSeq high-throughput sequencing followed by optimization yielded 2,507,118 ITS reads with a length of >400 bp from the 12 soil samples collected from four land-use types areas. After trimming and quality filtering, a total of 2,204,538 high-quality sequences with an average length of 449.75 bp were obtained. The shared communities in different land-use types were further determined via the Venn diagram (Fig. 1). The data clearly demonstrated that 2796 bacterial OTUs were common for all four land-use types, that 532-2316 bacterial OTUs were shared by any three land-use types and that 1311-5000 bacterial OTUs were shared by any two land-use types.

![Fig.1 Venn diagram for bacterial OTUs found in the four land-use types.](image)

3.2 Bacterial richness and diversity indices
Land use can alter soil chemical and physical properties, and its effect on soil bacterial diversity is shown in Table 2. The total number of OTUs obtained from the soil samples in FL, SL, GL and CL areas were 57400, 39500, 34350 and 41600, respectively. The Good’s coverage values fluctuated between 92.1% and 95.2%, indicating that the sequencing depth was sufficient to capture the diversity. Both the Ace and Chao1 indices were used to estimate the total number of species in the samples; these indices indicate bacterial richness. The Shannon and Simpson indices were employed to estimate soil microbial diversity. Chao1 of different land-use types varied from 17500 to 28267 in the 0–10 soil layer and decreased in the following order: FL>CL>SL>GL. And Shannon's index followed similar patterns with Chao1. These results indicated that the land-use system also influenced the bacterial richness and diversity in the analyzed samples, with lower values for the native grassland site.
Table 2: Characteristics of soil bacterial richness and diversity indices in different land-use types.

| Bacterial richness and diversity index | Land-use types |
|--------------------------------------|----------------|
|                                      | FL  | SL  | GL  | CL  |
| Total number of OTUs                 | 57,400 | 39,500 | 34,350 | 41,600 |
| Good's coverage                      | 0.921±0.004c | 0.942±0.006ab | 0.952±0.009a | 0.930±0.003bc |
| Chao1                                | 28267±1534a | 20300±1752ab | 17500±3011b | 20933±4125ab |
| Shannon                              | 1.161±0.008a | 1.067±0.023b | 0.999±0.025c | 1.095±0.012b |

Note: Mean ± SE (n=3)

Beta diversity analysis showed dissimilarity values among the different land-use types based on the biological distances. The results indicated that the degree of bacterial community dissimilarity among different land-use types was different. The Bray-Curtis distances were higher than 0.420 (between FL and CL, GL, SL) and 0.420 (between SL and GL, CL). In addition, the Bray-Curtis distance between GL and CL from 0.423 (between GL and CL) (Fig. 2).

3.3 Bacterial community composition

All valid sequences from the soil sample libraries were classified from genera to species based on the Unite Blast database. The results showed differences in the abundance of bacterial community at the genus level in four land-use types (Fig. 3). For FL, Proteobacteria made up the largest percentage, followed by Acidobacteria, Nitrospirae, Actinobacteria, Thaumarchaeota, Chloroflexi, Gemmatimonadetes, Latescibacteria. The relative abundance of Acidobacteria was the highest in SL, GL and CL. In all, the dominant genera were Actinobacteria and Proteobacteria except for GL. Marked differences in the distribution of taxon assemblages among the different land-use types in soil were found. The relative abundances of Acidobacteria in SL, Proteobacteria in FL, and Chloroflexi in GL presented as being higher than in other land-use types.
Fig. 3 The abundance of bacterial community at the genus level in four land-use types

3.4 Correlation between community structure and environmental factors
The relative abundances of Flavobacterium, Sphingomonas, Blastocatella, Zymomonas, Pedomicrobium and Reyranella were significantly positively correlated with pH. The relative abundance of Gemmatimonas, Rhodanomonas and Flavisolibacter had a marked correlation with TP. The relative abundance of Candidatus.Koribacter and Telmatobacter exhibited a negatively significant correlation with LAP (Fig. 3).

Fig. 4. Pearson’s correlation between the relative abundances of the abundant bacterial genera and the soil physicochemical properties.

4. Conclusion

4.1 The dominant taxonomic genera were Actinobacteria and Proteobacteria.

4.2 Land-use type influenced the bacterial richness and diversity, and ranked as follows: FL>CL>SL>GL.

4.3 Marked differences in the distribution of taxon assemblages among the different land-use types in soil were found. The relative abundances of Acidobacteria in SL, Proteobacteria in FL, and Chloroflexi in GL presented as being higher than in other land-use types.
Among all the physicochemical properties, significant Pearson linear correlations between soil bacterial taxonomic groups (Flavobacterium, Sphingomonas, Blastocatella, Zymomonas, Pedomicrobium, Reyranella, Rhodanomonas, Flavisolibacter, Candidatus and Telmatobacter) and physicochemical properties (pH, TP and LAP) were observed.

4.5 Land-use type is a major factor that affects the bacterial composition and diversity, and that changes in soil bacterial composition and diversity are associated with shifts in physicochemical properties.

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References
[1] Q.Y. Yang, Z.C. Jiang, Z.L. Ma, W.Q. Luo, Y.Q Xie, J.H. Cao. Earth. Sci. 70, 295-302 (2013)
[2] X.K. Qi, K.L. Wang, C.H. Zhang. Ecol. Eng 54, 245-253 (2013)
[3] D.J. Li, L. Wen, S. Jiang, T.Q. Song, K.L. Wang. J. Environ. Manage 207, 456-464 (2018)
[4] A.C.C. Ferreira, L.F.C. Leite, A.S.F. de Araújo, N. Eisenhauer. Land Degrad. Develop 27, 171-178 (2016)
[5] D. Liu, Y.M. Huang, S.S. An, H.Y. Sun, P. Bhopled, Z.W. Chen. Catena 162, 345-353 (2018)
[6] M. Maharjana, M. Sanaullah, B.S. Razavi, Y. Kuzyakov. Appl. Soil Ecol 113, 22-28 (2017)
[7] R. Almasia, M. Carú, M. Handford, J. Orlando. Soil Biol. Biochem 103, 39-45 (2016)
[8] S.D. Jurburg, T.N. da Luz, J. Raimundo, P.V. Morais, J.P. Sousa, J.D. van Elsas, J.F. Salles. Sci. Total Environ 618, 1638-1646 (2018)
[9] M.J. Yao, J.P. Rui, J.B. Li, J.M. Wang, W.D. Cao, X.Z. Li. Catena 165, 228-236 (2018)
[10] L.L. Ding, Y.S. Shang, W. Zhang, Y. Zhang, S.G. Li, X. Wei, X. Chen, Y.J. Zhang, X.L. Song, X. Chen, J.J. Liu, F.L. Yang, X.D. Yang, C. Zou, P.C. Wang*. Sci. Total Environ 709, 136207 (2020)