

## Rhizosphere Soil Microbial Community Diversity in Typical Tobacco-Growing Ecological Regions of Guizhou Province (Postprint)

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### Abstract

Soil microorganisms are important components of soil that play an active role in the formation of soil fertility and the transformation of plant nutrients. Using methods such as 16S V4 region high-throughput sequencing, we analyzed and studied the rhizosphere microbial communities of typical tobacco-planting soils in different ecological regions of Guizhou Province, elucidating the informational differences of typical soil rhizosphere microbial communities across different ecological regions from the perspective of rhizosphere soil microbial community characteristics. The results showed that weakly acidic and acidic soils accounted for 70.06% in different ecological regions of Guizhou Province, with an average organic carbon content of 2.24%, indicating generally high organic carbon content, and there was a certain correlation between total nitrogen and organic carbon content. The number of OTUs of rhizosphere soil microorganisms showed significant differences, generally presenting a pattern of western regions being higher than eastern regions, and southern regions being higher than northern regions. Differences in species abundance at the microbial genus level revealed that soil type had a significant effect on microbial community abundance. Among soil functional microorganisms, the relative abundance of microorganisms from the two phyla Proteobacteria and Actinobacteria was highest in the northern Guizhou region, while in the central Guizhou region, the abundance of Proteobacteria was significantly higher than that of Actinobacteria. In the southwestern and central Guizhou regions, the dominant populations of soil microorganisms at the genus level were significantly higher than in other regions, with low population balance, indicating potential risks for soil-borne disease occurrence. Through the analysis and research of rhizosphere microbial information of soil microorganisms in different ecological regions of Guizhou Province, a solid research foundation has been laid for subsequent soil bioremediation.

## Full Text

### Preamble

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### Investigation and Analysis of Microbial Information in Tobacco-Planted Soil from Different Ecological Regions in Guizhou Province

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### Abstract

Soil microorganisms are essential components of soil that play active roles in soil fertility formation and plant nutrient transformation. To elucidate differences in microbial community information among various ecological regions, we analyzed rhizosphere microbial communities in typical tobacco-planting soils from different ecological zones in Guizhou Province using high-throughput sequencing of the 16S V4 region. Results revealed significant variations in microbial communities across regions. Weakly acidic and acidic soils accounted for 70.06% of all samples, with average organic carbon content of 2.24%. Total nitrogen and organic carbon content showed positive correlation. Rhizosphere soil microbial quantities differed significantly, following a general pattern of higher abundance in western versus eastern regions and southern versus northern regions. Genus-level species abundance differences demonstrated that soil type significantly influenced microbial community richness. In terms of functional microorganisms, Proteobacteria and Actinobacteria were the most abundant phyla in northern Guizhou, while Proteobacteria abundance significantly exceeded that of Actinobacteria in central Guizhou. Southwest and central Guizhou regions exhibited significantly higher dominant populations at the genus level compared to other areas, but with low population balance, indicating potential risk for soil-borne disease outbreaks. This investigation of rhizosphere microbial information across different ecological regions in Guizhou establishes a solid foundation for future soil bioremediation research.

**Keywords:** high-throughput sequencing; soil microbial community; functional microorganisms; principal component analysis (PCA)

### Introduction

Soil microorganisms are key drivers of soil formation and transformation [1], playing irreplaceable roles in maintaining soil structure and influencing soil vegetation throughout soil development. They constitute indispensable indicators for soil quality evaluation [2]. Microbial diversity encompasses all microbial species in soil ecosystems, their genes, and the degree of diversification in their

interactions with the environment [3]. Functional diversity of soil microbial communities serves as an indicator of community status, reflecting ecological characteristics and soil fertility traits [4], which is crucial for understanding subsurface ecosystem structure and function and for conserving and utilizing microbial diversity resources [5]. Soil microbial community diversity primarily includes species diversity, genetic diversity, structural diversity, and functional diversity [6]. Rich microbial community diversity not only alleviates continuous cropping obstacles but also ensures stable soil health [7].

In recent years, researchers have increasingly recognized the important regulatory role of soil microorganisms in agricultural production, employing various techniques to enhance soil microbial activity. For instance, straw mulching improves soil microbial diversity [8], organic fertilizer application alters microbial community structure and improves metabolic function [9], and bio-organic fertilizer regulates microbial communities to achieve biological control of tobacco bacterial wilt [10]. Soil health, particularly microecological balance, is essential for producing high-quality tobacco leaves [11]. Previous studies have explored changes in rhizosphere microbial quantity, community functional diversity, and structural diversity following bio-organic fertilizer application [12], and Li et al. [13] demonstrated that integrated control measures balancing tobacco rhizosphere microbial communities significantly reduced bacterial wilt incidence. Wu et al. [13] investigated the effects of root exudates from different tobacco cultivars on microbial communities.

Globally, research on ecological environments and soil microorganisms has provided deeper insights into microbial composition, functional diversity, and spatiotemporal distribution [14]. The Global Soil Biodiversity Initiative launched in 2011 ([www.globalsoilbiodiversity.org](http://www.globalsoilbiodiversity.org)) aims to promote understanding of soil biodiversity and its ecological services, providing scientific basis for environmental policy formulation. Domestic monitoring and research on soil microbial diversity started relatively late in China, but technological advances have yielded fruitful results in microbial diversity and biogeography. Studies in China's black soil region revealed that bacterial community composition is primarily influenced by soil pH and organic carbon [15], while fungal community variation is mainly driven by soil organic carbon [16]. Research on soil bacteria [17] and fungi [18] diversity along elevation gradients on Changbai Mountain found that microbial diversity patterns differ from plants, with bacteria better reflecting plant and soil changes than fungi across elevations. Ren et al. [19] studied microbial flora on Taibai Mountain at different altitudes, but systematic analysis of microbial structure and diversity under comprehensive ecological factors, i.e., ecological zoning, remains lacking. The aforementioned studies focused on certain important factors in ecological environments rather than integrated ecological zoning.

The rapid development of next-generation high-throughput sequencing technology enables direct sequencing of millions to billions of gene sequences with high throughput and simultaneous analysis of hundreds of samples [20]. 16S rDNA sequencing is an important tool for analyzing species composition and relative

abundance of microbial communities in complex environments. Investigating soil microbial community dynamics is significant for regulating soil nutrient retention and meeting nutrient demands during different tobacco growth stages. Therefore, this study conducted comprehensive analysis of microbial communities in typical tobacco-planting ecological regions of Guizhou to provide theoretical basis for subsequent soil bioremediation.

## Materials and Methods

### Sample Collection

Rhizosphere soil samples were collected from typical tobacco-planting ecological regions in Guizhou Province after tobacco transplanting. Detailed records were made for each sample regarding sampling location and altitude. All collected soil samples came from tobacco fields with healthy plant growth and minimal disease incidence. The same type of organic fertilizer (oil cake organic fertilizer) was applied with reduced inorganic nitrogen. Five tobacco plants with consistent growth were selected from each plot. The entire root systems were excavated and gently shaken to remove bulk soil. Roots with attached soil were placed in 10 mL sterile water and ultrasonicated for 15 minutes at 55°C to obtain rhizosphere soil [21]. Basic information for the collected soil samples is presented in .

### Soil Physicochemical Analysis

Soil organic carbon content was determined using an Elementar high-precision elemental analyzer, and pH was measured using a FiveEasy Plus pH meter. provides an overview of the ecological regions, including sample numbers, locations, altitudes, and soil types across northwest, southwest, southeast, central, and north Guizhou regions.

### DNA Extraction and PCR Amplification

Soil DNA was extracted using the Soil DNA Isolation Kit (Omega). Appropriate amounts of sample were placed in centrifuge tubes and diluted with sterile water. Specific primers for the 16S V4 region and high-fidelity enzymes were used for amplification to ensure efficiency and accuracy. PCR was performed using Master Mix with GC Buffer (New England Biolabs) with barcode sequences. The primer pair 515F-806R was used for the target region.

The PCR reaction system consisted of 25  $\mu$ L Ex Taq loading buffer, 2  $\mu$ L DNA template, 1  $\mu$ L each of forward and reverse primers (25 pmol/ $\mu$ L), and 21  $\mu$ L ddH<sub>2</sub>O. PCR conditions were: initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were detected by agarose gel electrophoresis and purified using the GeneJET Gel Extraction Kit.

## Library Construction and Sequencing

Libraries were constructed using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs). Qualified libraries were sequenced on the MiSeq System (Illumina) using paired-end sequencing. Raw sequencing data were assembled to obtain effective data. UPARSE software (v7.0.1001) [23] was used for operational taxonomic unit (OTU) clustering and taxonomic classification based on effective data. Species annotation was combined with community structure statistics at various taxonomic levels to obtain OTU numbers and diversity indices for each sample. Principal component analysis (PCA) was performed based on OTU and species composition.

## Statistical Analysis

Experimental data were processed using Microsoft Excel 2003. Significance analysis was performed using SPSS Base Ver. 13.0 (SPSS, IL, USA). Multiple comparisons were conducted using Duncan's new multiple range test (LSD) at significance level  $P < 0.05$  [25].

## Results and Analysis

### 1. Basic Physicochemical Properties of Soil Samples from Different Tobacco-Planting Ecological Regions

Soil pH values ranged from 4.88 to 7.45. Acidic soils ( $\text{pH} > 7.5$ ) accounted for 29.04% of total samples, neutral soils ( $\text{pH} 6.5\text{-}7.5$ ) accounted for 35.48%, and strongly acidic soils ( $\text{pH} < 5.5$ ) accounted for 35.48% of total samples. Yellow soils were predominantly neutral and weakly acidic, with strongly acidic yellow soils comprising only 2.87% of total samples.

Soil organic carbon content was high, ranging from 1.11% to 3.69%. According to soil nutrient classification systems, 54.84% of samples had organic carbon content  $> 2.87\%$ . Organic carbon content showed an increasing trend with altitude. Total nitrogen content ranged from 0.14% to 0.34%, with 64.52% of samples having total nitrogen  $> 0.2\%$ . Yellow-brown soils and paddy soils had the highest total nitrogen content. No correlation was observed between total nitrogen content and altitude within regions. The northwest Guizhou region had relatively higher total nitrogen content compared to other regions.

### 2. Soil Microbial Species Diversity in Different Tobacco-Planting Ecological Regions

Across different ecological regions, soil microbial OTU numbers showed significant variation, generally following the pattern of western  $>$  eastern and southern  $>$  northern regions. Northern Guizhou exhibited the highest OTU numbers, followed by central Guizhou. The two sub-regions of central Guizhou (central and south-central) showed minimal difference in OTU numbers. Northwest Guizhou had the lowest OTU numbers.

Within the same ecological region, soil samples from different altitudes showed significant differences in OTU numbers. In southeast Guizhou, OTU numbers decreased with increasing altitude. In contrast, northern Guizhou showed the highest OTU numbers at medium altitude, followed by low altitude, with high altitude having the lowest. While no significant differences were observed between southeast and north Guizhou at high and low altitudes, northern Guizhou's medium-altitude OTU numbers were significantly higher (1.44 times) than those in southeast Guizhou.

Different soil types also showed varying microbial OTU numbers. Purple soils had significantly higher OTU numbers than yellow soils, which in turn were significantly higher than alluvial and coarse skeletal soils. Yellow-brown soils had the lowest microbial OTU numbers, at only 25.5% of those in purple soils and 59.5% of those in yellow soils.

### **3. Analysis of Soil Microbial Community Structure Diversity at Genus Level**

Based on microbial genus-level annotation and abundance information from all samples, dominant bacterial genera were identified in different regions. Tianzhu purple soil (TZZT) and Weining purple soil (WNZT) showed the most distant clustering relationships with other soil types across all ecological regions. Within the same ecological region, different altitudes showed varying microbial abundance clustering patterns. For example, in Tianzhu county, low-altitude soil (TZ350) clustered closely with medium-altitude soil (TZ550), but distantly from high-altitude soil (TZ750). In contrast, medium and high-altitude soils showed the closest clustering.

Among different soil types, Tianzhu purple soil (TZZT) showed the most distant clustering relationship with all other soil types at the genus level. Paddy soils from different regions showed relatively close clustering relationships, though Zunyi paddy soil (ZYS DT) and Dushan paddy soil (DSSDT) showed relatively distant clustering compared to other paddy soils.

### **4. Specific Functional Diversity of Soil Microorganisms in Different Tobacco-Planting Ecological Regions**

Analysis of microbial abundance revealed significant differences in microbial composition proportions across regions. Firmicutes were concentrated primarily in central Guizhou samples. Many Firmicutes can produce spores that resist desiccation and extreme environmental conditions, potentially mitigating weather-related impacts on soil [32]. The Gammaproteobacteria subphylum showed the highest proportion in Tianzhu purple soil (TZZT), which may provide resistance against soil-borne pathogens [33].

## 5. Principal Component Analysis of Rhizosphere Soil Microbial Abundance and Soil Physicochemical Properties

PCA revealed that microbial communities from the same region clustered closely together, indicating similar community composition within regions. However, individual samples from southwest Guizhou showed greater dispersion. Northern Guizhou samples exhibited distinct inter-sample differences. In central Guizhou, microbial communities were strongly influenced by soil nutrient elements, with significant differences in community composition structure among soils. In other regions, nutrient influence was less pronounced and community composition was more similar.

The first principal component (PC1) and second principal component (PC2) explained 20.80% and 13.78% of the variance, respectively, representing the two major factors differentiating soil microbial community structures across ecological regions. This indicates that soil microbial composition is determined not by single factors but by combined effects of multiple factors including soil nutrient status and soil type categories.

## Discussion and Conclusion

Soil microbial indicators primarily include microbial quantity, diversity, and activity, which directly or indirectly determine soil physicochemical properties and nutrient availability. Microbial diversity is the most critical factor for soil stability and quality [26], capable of regulating various soil abiotic indicators. Therefore, studying soil microbial diversity and population structure has become a paramount field in soil health and fertility research.

In this study, strongly acidic soils (pH < 5.5) accounted for 35.48% of samples from Guizhou's typical tobacco-planting regions. Soil pH is negatively correlated with disease suppression capacity—more acidic soils exhibit weaker disease suppression [27-28]. Under acidic conditions with abundant organic carbon and total nitrogen, soil-borne disease outbreaks are highly likely, providing early warning of microbial community imbalance from physicochemical indicators.

In central Guizhou, soil microorganisms were strongly influenced by nutrient elements, with significant differences in community composition structure among different soils. In other regions, nutrient influence was less pronounced and community composition was more similar, suggesting that microbial communities may be more closely related to ecological conditions than nutrient status [29].

The overall trend of OTU numbers (western > eastern, southern > northern) aligns with temperature patterns during tobacco transplanting season. Microbial community balance is a crucial indicator of soil health. Imbalanced communities with dominant populations and reduced diversity and evenness can lead to transition from bacterial-dominated to fungal-dominated soils, increasing pathogen populations such as *Ralstonia solanacearum* [11].

Purple soils consistently harbored dominant populations and showed the most

distant clustering relationships with other soil types, indicating substantial influence of soil type on microbial community diversity—a finding similar to Perez et al. [30]. Paddy soils showed relatively close clustering relationships in terms of microbial abundance.

The concentration of Firmicutes in central Guizhou samples is noteworthy, as many Firmicutes produce spores that resist environmental stress [32]. The high proportion of Gammaproteobacteria in Tianzhu purple soil (TZTZ) may provide potential resistance against soil-borne pathogens [33].

Previous research in Guizhou's different ecological regions has focused on tobacco leaf quality [34], nitrogen application rates [35], and organic fertilizer effects [36], with no systematic studies on microbial flora. The maturation and popularization of second-generation high-throughput sequencing technology now enable deep sequencing of environmental microbes, overcoming cultivation limitations, objectively revealing microbial community structure, and sensitively detecting subtle changes in response to environmental variations. This has important theoretical and practical significance for studying microbe-environment relationships, environmental remediation, and microbial resource utilization.

Shen et al. [17] found that soil bacterial quantity is positively correlated with soil organic matter and total nitrogen content, similar to our finding that microbial diversity indices correlate with organic matter content. PCA effectively extracts major elements and structures from multidimensional data, providing more informative analysis than simple species abundance clustering [39]. Our PCA confirmed that PC1 and PC2 represent the two major differentiating factors for soil microbial community structures across ecological regions, with contribution rates of 20.80% and 13.78%, respectively, demonstrating that microbial composition is determined by comprehensive factors rather than single variables.

In conclusion, this analysis of rhizosphere microbial communities in typical tobacco-planting ecological regions of Guizhou provides a theoretical foundation and establishes a solid research basis for subsequent soil bioremediation.

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