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Effects of Soil Physicochemical Properties on Bacterial Community Dynamics in Grassland Ecosystems

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Abstract

This study investigated the effects of soil physicochemical properties on bacterial community structure and function in tobacco rhizosphere (GJ) and bulk soil (GW), aiming to elucidate key environmental drivers and their mechanisms. Field experiments were conducted in five-year continuous tobacco cultivation fields in Lichuan City, Hubei Province. Soil samples were collected from healthy (JK) and diseased (FB) plants at 45, 75, and 105 days post-transplantation. Soil parameters, including pH, organic matter (OM), macronutrients (AN, P, K), and micronutrients (Fe, Mn) were analyzed. Bacterial communities were characterized via 16S rRNA gene sequencing, and interactive effects of microhabitat, soil status, and sampling time were assessed using linear mixed-effects models (LMMs). Results demonstrated that microhabitat exerted the strongest influence on bacterial diversity ($\beta = 1.70$ - 1.83, $p < 0.0001$), surpassing soil type and sampling time. Rhizosphere soils exhibited significant increases in AN, K, pH, TN, Ca, Mg, and Mn, but decreases in P, OM, Fe, and Cu. Microhabitat differentiation elevated the abundance of beneficial phyla including Chloroflexi ($\beta = 1.9$), Actinobacteria ($\beta = 1.7$), and Bacteroidetes ($\beta = 1.3$), while reducing Proteobacteria ($\beta = -1.6$), Cyanobacteria ($\beta = -0.8$), and Firmicutes ($\beta = -0.5$). At the genus level, Bacillus ($\beta = 0.35$) and Streptomyces ($\beta = 0.96$) were enriched, whereas Pseudomonas ($\beta = -1.27$) was depleted. Mantel tests revealed significant correlations between Fe, Mn, P, and microbial composition ($r = 0.42$ - 0.58, $p < 0.01$), suggesting micronutrients drive community assembly through direct metabolic regulation or indirect environmental modulation. These findings highlight that soil microhabitats critically shape root-associated microbial diversity and functional guilds via phys-

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icochemical property mediation, providing a theoretical foundation for optimizing soil health management and microbial ecological regulation in tobacco production systems.

Keywords

Soil Physicochemical Properties, Tobacco, Root-Associated Microbiome, Linear Mixed-Effects Models

1. Introduction

Tobacco (*Nicotiana tabacum* L.), a globally cultivated economic crop, holds significant agricultural importance [1]. Its growth and yield are closely associated with soil physicochemical properties and dynamic plant-microbe interactions, particularly in rhizosphere and bulk soil bacterial communities [2]. The rhizosphere, a narrow soil zone directly influenced by root exudates, harbors bacteria that enhance nutrient acquisition, pathogen suppression, and stress tolerance through symbiotic relationships [3]-[5]. In contrast, the rhizoplane (root surface microbiome), serving as the primary interface for microbial colonization, mediates plant-microbe communication [6] [7]. Despite the critical ecological functions of these microbial niches, the mechanisms by which soil physicochemical properties regulate their structural dynamics remain poorly understood, particularly under long-term monoculture and climate change scenarios.

Previous studies have highlighted the impacts of soil pH, organic matter (OM), and macronutrients (e.g., nitrogen, phosphorus, potassium) on microbial diversity [8] [9]. For instance, Lauber *et al.* (2009) demonstrated strong correlations between soil pH and bacterial community composition [10], while Shi *et al.* (2021) identified carbon availability as a key driver of rhizosphere microbiome assembly [11]. However, existing research predominantly focuses on model plants (e.g., Arabidopsis, rice), with limited attention to economically significant crops like tobacco. Furthermore, the ecological functional divergence between rhizosphere and bulk soil microbiomes and their response mechanisms to soil variables remain unresolved. Although Bulgarelli *et al.* (2013) revealed compartment-specific microbial recruitment patterns in barley roots [12], analogous mechanisms in field-grown tobacco systems await exploration. Previous studies on rhizosphere microbiome assembly have predominantly focused on model plants such as Arabidopsis and rice (e.g., Bulgarelli *et al.*, 2013), with limited attention to economically significant crops under long-term monoculture regimes. For instance, while Xiao *et al.* (2016) explored microbial dynamics in Panax ginseng systems, analogous investigations in tobacco cultivation—a crop subjected to intensive fertilization and pathogen pressures—remain scarce [13]. This study addresses this critical gap by elucidating how soil physicochemical properties mediate microbial community dynamics in tobacco rhizosphere and bulk soil, particularly under continuous cultivation and climate change scenarios.

Global climate change and agricultural intensification have exacerbated soil degradation, weakened microbial community resilience, and threatened crop sustainability. In tobacco cultivation systems, prolonged monoculture and excessive fertilization induce soil acidification, nutrient imbalance, and pathogen accumulation (e.g., *Ralstonia solanacearum*), leading to frequent outbreaks of bacterial wilt [14]. These challenges underscore the urgency to elucidate how soil physicochemical properties regulate tobacco-associated microbiomes, thereby informing precision soil management strategies.

Under escalating environmental stresses driven by climate change and shifting land-use practices, understanding the impacts of soil physicochemical variations on tobacco rhizosphere and bulk soil bacterial communities is critical for preserving soil ecological functions and promoting sustainable tobacco production. This study investigates the effects of soil physicochemical properties on bacterial communities in tobacco rhizosphere and bulk soil, identifies key determinants, and deciphers their interaction mechanisms. By comparative analysis of bacterial communities under varying soil conditions, this work advances soil microbial ecology research and provides scientific foundations for ecological regulation and soil health management in tobacco production systems.

2. Materials and Methods

2.1. Study Site and Sampling

This study was conducted in tobacco fields with over five years of continuous cultivation in Lichuan City, Hubei Province (latitude N, longitude W). The experimental design focused on root-associated microbial communities and soil physicochemical properties (GJ: rhizosphere; GW: bulk soil). Healthy (JK) and diseased (FB) tobacco plants were sampled at 45, 75, and 105 days post-transplantation. The study area exhibits a temperate climate with a mean annual temperature of 16.7˚C and an average annual precipitation of 1304 mm. Soil samples were collected from the rhizosphere (0 - 20 cm depth) and bulk soil (20 - 40 cm depth) using a stainless steel auger. For each treatment combination (healthy/diseased plants × three sampling times), five biological replicates were collected, resulting in a total of 120 samples (2 microhabitats × 2 soil statuses × 3 time points × 5 replicates). Healthy plants (JK) were defined as those showing no visible disease symptoms (e.g., wilting, chlorosis), whereas diseased plants (FB) exhibited characteristic bacterial wilt symptoms, including leaf yellowing and vascular browning. Sampling time points (45, 75, and 105 days post-transplantation) were selected to capture critical growth stages: early vegetative growth, flowering, and maturation.

2.2. Field Measurements and Soil Chemical Analyses

Prior to chemical and microbial analyses, visible stones and plant roots were removed from soil samples using sterile metal tweezers. Soil physicochemical properties were determined as follows:

pH: 5 g of air-dried soil was mixed with 25 mL of deionized water, agitated for

30 min, and measured using a pH meter [15].

Organic matter (OM): Quantified via potassium dichromate oxidation [16]. Briefly, 1 g soil was reacted with 20 mL 0.8 mol/L $K_2Cr_2O_7$ and 10 mL concentrated H_2SO_4, boiled, cooled, diluted to 100 mL, and analyzed spectrophotometrically at 620 nm.

Total nitrogen (TN): Determined by Kjeldahl digestion [17]. Soil was digested with H_2SO_4 and K_2SO_4, distilled, and ammonia was absorbed in boric acid for titration.

Available phosphorus (AP): Extracted with 0.5 M $NaHCO_3$ and measured using the molybdenum-antimony colorimetric method at 700 nm [18].

Available potassium (AK): Analyzed by flame photometry after extraction with 1 M ammonium acetate [19].

2.3. DNA Extraction

To ensure high-quality sequencing data, DNA was extracted using a protocol combining mechanical grinding, freeze-thaw cycles, and SDS-based cell lysis [20]. Purification was performed using a commercial DNA extraction kit following the manufacturer's protocol. DNA quality was assessed via NanoDrop ND-1000 spectrophotometer (260/280 > 1.8; 260/230 > 1.7), and concentrations were quantified using a Qubit fluorometer. DNA samples were stored at −80˚C until further processing.

2.4. Amplicon Sequencing and Data Preprocessing

A two-step PCR protocol was employed to prepare 16S rRNA gene libraries, minimizing amplification bias [21] [22].

Primary PCR: 10 ng DNA per sample was amplified in triplicate (25 μL reactions, 10 cycles) using primers without adapters. Products were pooled, purified, and eluted in 50 μL deionized water.

Secondary PCR: 15 μL of primary PCR product was amplified (15 cycles) with barcoded primers containing full adapters. Low total cycles (25 - 30) ensured unsaturated amplification and minimized artifacts.

Purified amplicons were sequenced on an Illumina MiSeq platform (2 × 250 bp kit) at the University of Oklahoma's Environmental Genomics Institute. Primer sequences were trimmed from paired-end reads using FLASH [23]. Merged reads containing ambiguous bases or shorter than 245 bp were discarded. High-quality sequences were processed into amplicon sequence variants (ASVs) via UNOISE3 [24].

Representative 16S rRNA sequences were aligned with Clustal Omega v1.2.2 and phylogenetically analyzed using FastTree2 v2.1.10 under Silva Living Tree Project constraints [25]. Taxonomic classification was performed using the RDP classifier (50% confidence threshold) [26] [27], with chloroplast and mitochondrial sequences removed.

Bioinformatics: Raw sequences were processed using FLASH v1.2.11 (Magoč

& Salzberg, 2011) with a minimum overlap of 20 bp. ASV clustering was performed via UNOISE3 (Edgar, 2016) with a 97% similarity threshold. Taxonomic classification utilized the SILVA v138 database [28] in QIIME2 v2021.4 [29].

2.5. Statistical Analyses

2.5.1. Diversity Analysis

Taxonomic α-diversity (richness, Shannon index) was calculated using the vegan R package [30].

2.5.2. Treatment Effects by LMMs

Linear mixed-effects models (LMMs) implemented in the lme4 R package [31] evaluated treatment effects on bacterial diversity and relative abundance. Fixed effects included microhabitat (GJ/GW), soil status (JK/FB), sampling time (Day_045/075/105), and their interactions. Random effects accounted for block design: y ~ Microhabitat × Type × Time + (1|Location)

3. Result

3.1. Assessment of Soil Physicochemical Properties on Root Microbial Communities Using Linear Mixed-Effects Models

3.1.1. Independent Effects of Soil Physicochemical Properties

Linear mixed-effects models (LMMs) revealed significant impacts of soil physicochemical properties on microbial diversity. Compared to bulk soil (GW), rhizosphere (GJ) soils exhibited elevated ammonium nitrogen (AN: $\beta = 1.71$), available potassium (K: $\beta = 1.82$), pH ($\beta = 1.82$), total nitrogen (TN: $\beta = 1.75$), calcium (Ca: $\beta = 1.72$), magnesium (Mg: $\beta = 1.73$), and manganese (Mn: $\beta = 1.70$) ($p < 0.0001$; **Figure 1**), alongside reduced available phosphorus (P: $\beta = -1.25$), organic matter (OM: $\beta = -0.44$), iron (Fe: $\beta = -0.89$), and copper (Cu: $\beta = -0.53$) ($p < 0.05$; **Figure 2**). The strongest effects were observed for pH ($\beta = 1.82$) and K ($\beta = 1.71$), suggesting rhizosphere acidification alleviation and potassium enrichment may directly drive microbial community restructuring.

3.1.2. Microhabitat Differentiation (GJ vs. GW)

Significant divergence in microbial diversity was observed between rhizosphere (GJ) and bulk soil (GW). GJ soils showed higher bacterial richness ($\beta = 1.71$), Shannon diversity ($\beta = 1.82$), and Pielou's evenness ($\beta = 1.81$) compared to GW ($p < 1.0 \times 10^{-25}$; **Table 1**). At the phylum level, GJ soils were enriched in Chloroflexi ($\beta = 1.9$), Actinobacteria ($\beta = 1.7$), and Bacteroidetes ($\beta = 1.5$), while Proteobacteria ($\beta = -1.6$) and Firmicutes ($\beta = -0.58$) were depleted ($p < 0.05$; **Figure 2E**). These results indicate rhizosphere environments selectively enrich functional phyla, forming distinct microbial functional units.

3.1.3. Temporal Dynamics (Sampling Day Effects)

Bacterial richness ($\beta = -0.44$) and Shannon diversity ($\beta = -0.16$) declined significantly from Day_045 to Day_105 ($p < 0.05$; **Table 1**). Chloroflexi abundance decreased by 12% in GJ soils at Day_105 compared to Day_045, while Actinobacteria

increased by 8% (Figure 2E). These temporal shifts may reflect seasonal variations in root exudate composition.

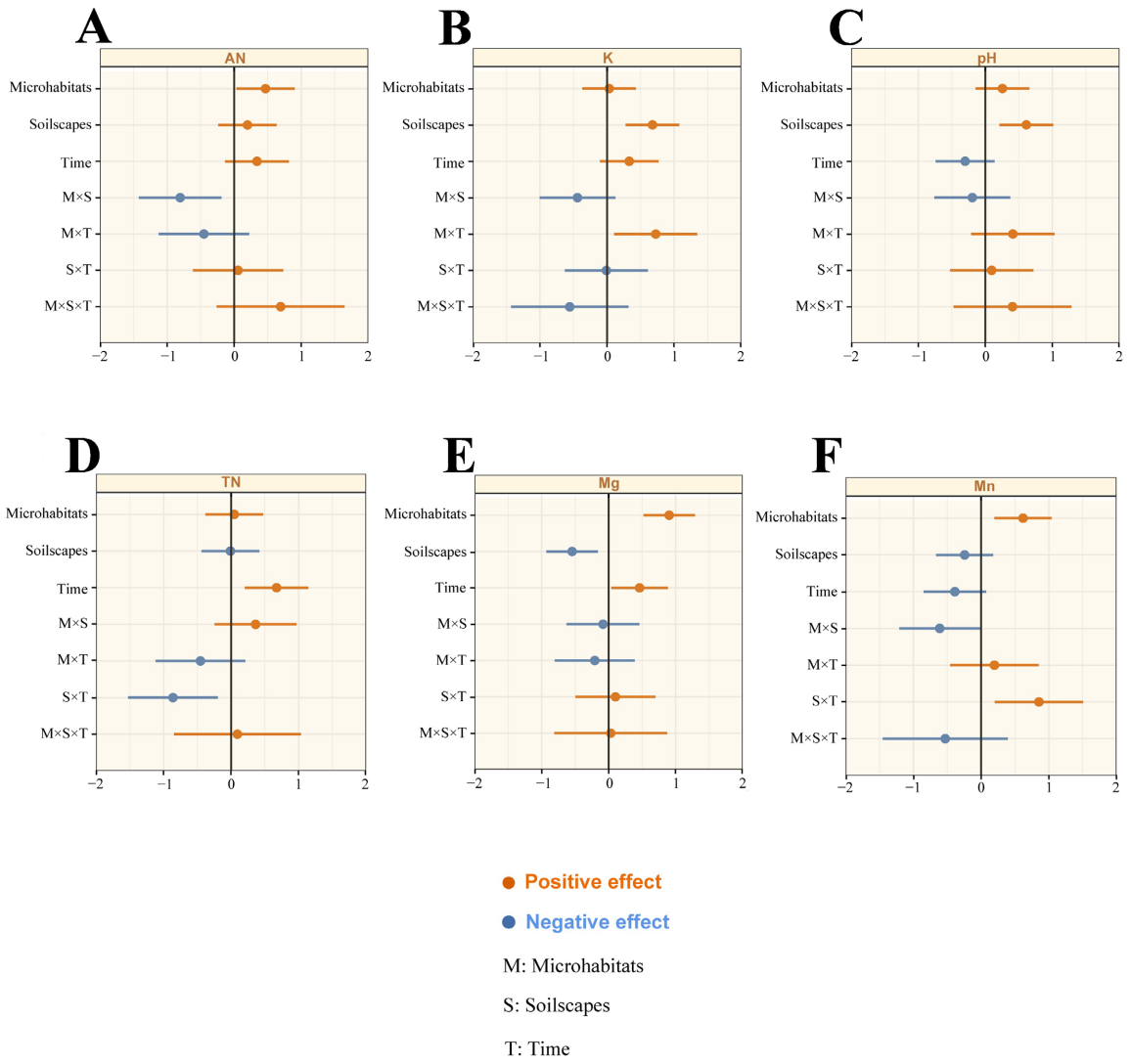

Figure 1. Positive influence of experimental treatments on soil and plant variables by linear mixed-effects models (LMMs). A, AN; B, K; C, pH; D, TN; E, Mg; F, Mn. Data are presented as mean values ± standard errors of the estimated effect sizes.

Table 1. Treatment effects on microbial diversity based on linear mixed-effects models.

Treatment Effects		Richness	Shannon	Simpson	Pielou_evenness
Microhabitats	β	1.70592	1.822881	−1.24739	1.81266
	t	8.683633	9.275137	−4.08459	8.517867
	p	1.46E−87	2.78E−90	8.92E−25	4.72E−74
Soilscapes	β	0.135675	−0.08266	0.47317	−0.18849
	t	0.690626	−0.42059	1.549398	−0.88575
	p	0.678875	0.910362	0.303438	0.67498

Continued

Time	β	−0.4408	−0.15611	0.298149	−0.03939
	t	−2.04829	−0.7251	0.891227	−0.16895
	p	2.40E−05	0.01948	0.128176	0.139347
M × S	β	−0.05243	0.093617	−0.4301	0.183652
	t	−0.18872	0.336823	−0.99586	0.610232
	p	0.797522	0.430084	0.091994	0.24957
M × T	β	0.118671	−0.24267	0.147794	−0.37935
	t	0.389924	−0.79701	0.312389	−1.15067
	p	0.558146	0.358762	0.725094	0.140217
S × T	β	−0.15353	0.006828	−0.205	0.078016
	t	−0.50445	0.022426	−0.4333	0.236644
	p	0.496988	0.809451	0.482116	0.62628
M × S × T	β	0.014703	0.090172	−0.0603	0.07101
	t	0.034161	0.209418	−0.09013	0.152305
	p	0.972749	0.834122	0.928188	0.878947

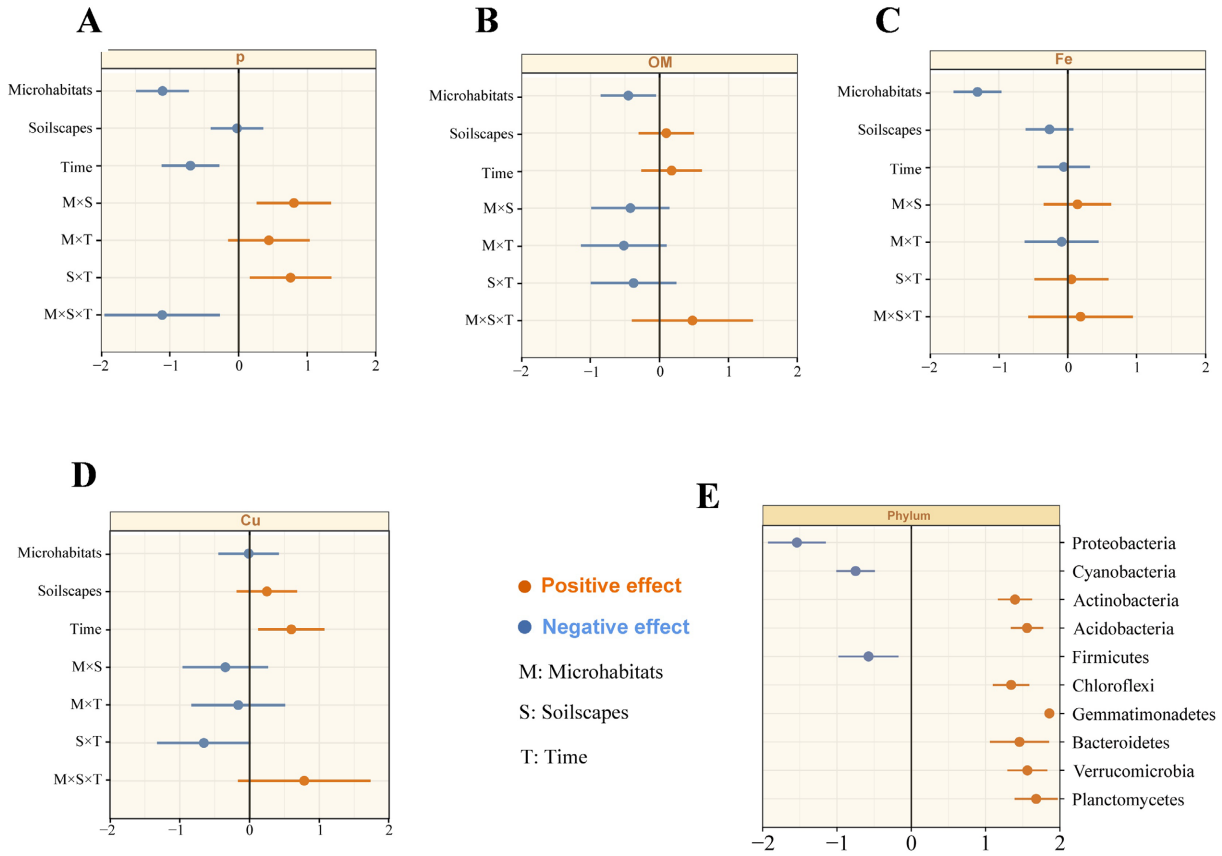

Figure 2. Negative influence of experimental treatments on soil and plant variables by linear mixed-effects models (LMMs) and LMMs β-effect values of the top 10 bacterial phyla. A, P; B, OM; C, Fe; D, Cu. E, LMMs β-effect values of the top 10 bacterial phyla. Data are presented as mean values ± standard errors of the estimated effect sizes.

3.1.4. Plant Health Status (JK vs. FB)

Differences between healthy (JK) and diseased (FB) rhizosphere communities were marginal. Although FB soils showed slight increases in pathogenic phyla (e.g., Proteobacteria: $\beta = 0.47$), overall diversity indices (e.g., Shannon: $\beta = -0.08$) and physicochemical parameters (e.g., OM: $\beta = -0.19$) remained non-significant ($p > 0.05$; Table 1). However, genus-level analysis revealed a significant depletion of Pseudomonas in FB soils ($\beta = -1.27$, $p < 0.001$; Figure 3B), potentially linked to competitive inhibition against *Ralstonia solanacearum*.

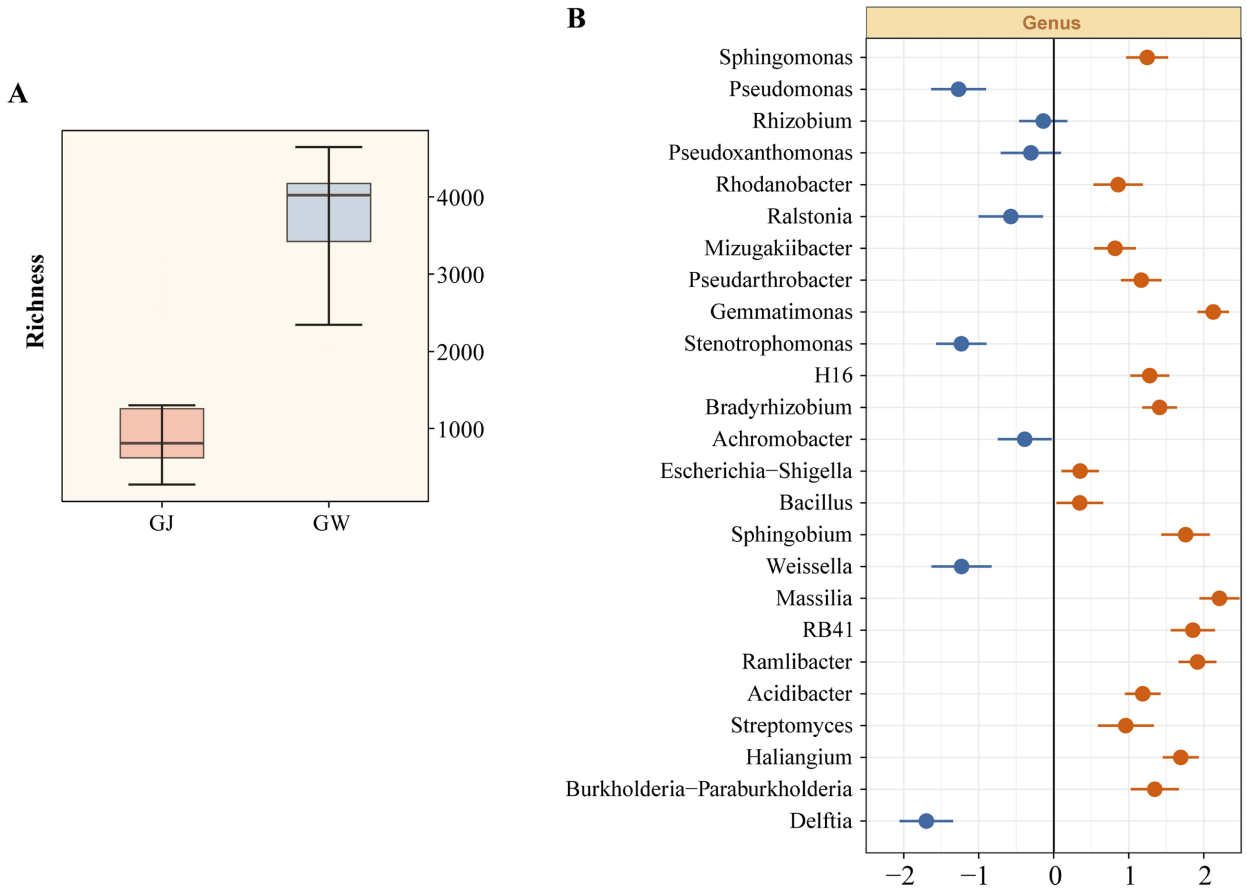

Figure 3. Effects of experimental treatments on bacterial diversity by linear mixed-effects models (LMMs). A, Bacterial alpha diversity; B, LMMs β-effect values of the top 25 bacterial genera.

3.1.5. Multi-Factor Interactions

Interaction analysis revealed weak negative effects of microhabitat × time (M × T) on Simpson index ($\beta = -0.38$, $p = 0.14$), whereas soil status × time (S × T) interactions were non-significant ($p > 0.48$; Table 1). Notably, GJ soils in healthy plants (JK) exhibited stronger enrichment of beneficial taxa (e.g., Streptomyces: $\beta = 0.96$), peaking at Day_75 (Figure 3B). Mantel tests further identified Fe ($r = 0.32$, $p = 0.01$) and Mn ($r = 0.28$, $p = 0.03$) as key correlates of community structure (Figure 4), suggesting micronutrients indirectly modulate community assembly via redox state regulation.

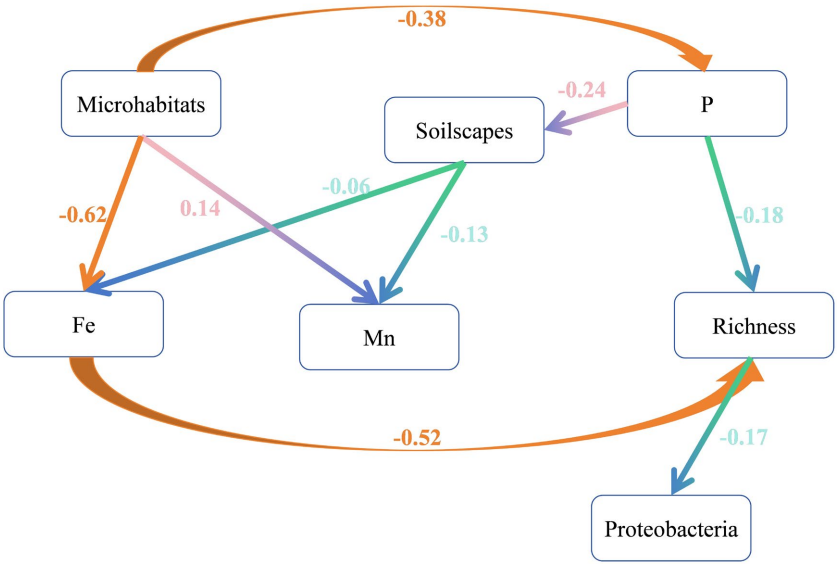

Figure 4. Mantel test correlation analysis model.

3.2. Impacts of Microhabitats on Microbial Biodiversity

Operational taxonomic unit (OTU) analysis (average 50,000 reads/sample) demonstrated that microhabitat exerted the strongest positive effects on richness (β = 1.70), Shannon index (β = 1.83), and Pielou's evenness (β = 1.81) ($p < 0.0001$), with effect magnitudes 3 - 60 times greater than sampling time (Table 1). Interaction effects between microhabitat, soil status, and time were negligible (β = 0.01 - 0.09), indicating microbial diversity shifts were mediated indirectly through physicochemical property alterations rather than direct treatment impacts.

Microhabitat-driven diversity modulation exhibited lineage-specific patterns. Proteobacteria (β = −1.6), Cyanobacteria (β = −0.8), and Firmicutes (β = −0.5) were significantly depleted in GJ soils ($p < 0.05$), while Chloroflexi (β = 1.9), Actinobacteria (β = 1.7), Bacteroidetes (β = 1.5), Acidobacteria (β = 1.4), Verrucomicrobia (β = 1.3), Planctomycetes (β = 1.2), and Gemmatimonadetes (β = 1.1) were enriched. Notably, GJ soils reduced phylum-level richness of the pathogen *Ralstonia solanacearum* while increasing beneficial taxa (e.g., Streptomyces; Figure 3A). Paradoxically, Firmicutes (containing antagonistic Bacillus spp.) were depleted in GJ (β = −0.58, $p < 0.01$), highlighting niche-specific trade-offs in functional guild recruitment.

Genus-level analysis of the top 25 taxa (Figure 3B) revealed significant enrichment of Bacillus (β = 0.35, $p < 0.001$) and Streptomyces (β = 0.96, $p < 0.001$) in GJ soils, alongside depletion of Pseudomonas (β = −1.27, $p < 0.001$). These results suggest microhabitat primarily modulates functionally distinct microbial consortia rather than broad taxonomic groups.

3.3. Interaction between Physicochemical Properties and Root-Associated Microbes

Mantel tests (Figure 4) identified significant correlations between selected physi-

cochemical properties (P, Fe, Mg, Mn; $|\beta| > 0.5$, $p < 0.05$) and representative bacterial genera (Massilia, Gemmatimonas, Ramlibacter, RB41, Sphingobium, Haliangium, Delftia; $|\beta| > 1.5$, $p < 0.05$). Micronutrients Mg and Mn exhibited strong associations with rhizosphere-enriched taxa ($r = 0.24$ - 0.38, $p < 0.05$), potentially driving beneficial microbial recruitment through direct metabolic stimulation (e.g., enzyme cofactor roles) or indirect environmental modulation (e.g., redox potential shifts). These interactions underscore the dual role of micronutrients in shaping rhizosphere microbiome assembly.

4. Discussion

This study provides a comprehensive investigation into the effects of soil physicochemical properties on bacterial community structure and function in tobacco rhizosphere and bulk soil, with comparative analyses against existing literature. Our results demonstrate that soil microhabitat is the dominant factor influencing root-associated microbial diversity, exhibiting significantly greater effect sizes than soil type or sampling time. Furthermore, microhabitat differentiation profoundly altered the abundance of specific bacterial phyla, with potential cascading effects on ecosystem functioning and plant health.

The positive regulation of bacterial diversity by microhabitat aligns with findings by Shi *et al.* (2018) [32], who reported soil microhabitat as a critical determinant of community diversity. However, our study advances this understanding by revealing microhabitat-driven shifts in phylum-level abundances—a phenomenon underexplored in prior work. This divergence likely stems from our refined sampling strategy and advanced molecular techniques, which enhanced resolution in assessing microhabitat effects.

Our findings resonate with Xiao *et al.* (2016), who emphasized the pivotal roles of soil pH and organic matter in shaping rhizosphere communities. Through linear mixed-effects models (LMMs), we further disentangled the complex interactions among microhabitat, soil status, and temporal dynamics, offering novel insights into their synergistic regulation of microbial diversity.

In contrast to Carey *et al.* (2016) [33], who attributed climate warming-induced microbial diversity loss primarily to soil temperature increases, our work highlights the central role of physicochemical properties in mediating community assembly. This distinction carries critical implications for predicting soil microbial responses to global change, particularly under long-term climatic stressors.

From an agricultural perspective, these findings hold practical significance. Targeted manipulation of key physicochemical factors (e.g., pH optimization, organic matter management) could enhance beneficial bacterial recruitment, thereby improving disease resistance and nutrient use efficiency in tobacco production systems.

This study focused solely on bacterial communities, while soil microbiomes encompass fungi and other microbial guilds. Future work should expand to these taxa for a holistic understanding. Additionally, our geographically restricted sam-

pling (Hubei Province, China) necessitates validation across broader pedoclimatic regions to assess generalizability.

The enrichment of Actinobacteria and Chloroflexi in rhizosphere soils suggests enhanced nutrient cycling and stress tolerance potential, which could be leveraged to reduce dependency on synthetic fertilizers in tobacco production. Furthermore, the depletion of Pseudomonas in diseased soils highlights the need for targeted biocontrol strategies, such as augmenting antagonistic Bacillus spp., to mitigate bacterial wilt outbreaks. These findings align with global efforts to promote sustainable agriculture through microbiome engineering [34], offering actionable insights for soil health management in monoculture systems worldwide.

5. Conclusion

This study elucidates the mechanisms by which soil physicochemical properties regulate bacterial communities in tobacco rhizosphere and bulk soil. Key findings include: Soil microhabitat exerted the strongest influence on bacterial diversity, surpassing soil type and temporal effects. Microhabitat differentiation significantly enriched beneficial phyla (Chloroflexi, Actinobacteria, Bacteroidetes) while depleting Proteobacteria, Cyanobacteria, and Firmicutes. Functional microbial consortia, rather than broad taxonomic groups, were preferentially modulated by microhabitat. These results advance soil microbial ecology by clarifying niche-specific recruitment mechanisms and provide actionable insights for optimizing soil health management in tobacco cultivation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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