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Effects of microbial organic fertilizers on soil microbial communities and physicochemical properties in tobacco cultivation

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Introduction: Tobacco, as an economic crop in our country, not only requires changes in cultivation practices but also benefits from reasonable fertilization to enhance yield and quality. Microbial organic fertilizers (MOFs) have received considerable attention in recent years; however, the relationship between the rhizosphere soil microorganisms of tobacco plants and the physicochemical properties of soil with MOFs remains poorly understood.

Methods: This study established experimental and control groups, employing a five-point sampling method to collect samples of the rhizosphere soil and tobacco leaves at four different growth stages. High-throughput 16S rRNA gene sequencing was conducted to analyze the microbial community structure, while the physicochemical properties of the soil and the physical characteristics of the tobacco leaves were also examined.

Results and Discussion: The results indicated that MOF reduced the uptake of ammonium nitrogen by tobacco at different growth stages, promoted the dispersion of the microbial community, and significantly altered the soil microbial community structure. At the phylum level, *Actinobacteriota* and *Proteobacteria* are the first and second dominant bacterial phyla in the soil microbial community. Significantly, during the prosperous growth stage of tobacco, the relative abundance of *Actinobacteriota* (14.90%) in the control group was lower than that of *Proteobacteria* (19.87%), whereas in the experimental group, the relative abundance of *Actinobacteriota* (22.99%) was higher than that of *Proteobacteria* (22.06%). Furthermore, an assessment of tobacco leaf morphology, yield, and quality demonstrated that the application of MOF resulted in a 21.93% increase in leaf yield and a 27.38% increase in yield value per unit area. Furthermore, nicotine and nitrogen content in the tobacco leaves slightly decreased. In summary, this study demonstrates that MOF can

improve soil microbial communities and enhance the quality and yield of tobacco leaves, providing valuable insights into the effects of MOF on altering the physicochemical properties of tobacco soil and improving leaf quality.

KEYWORDS

microbial organic fertilizer, tobacco cultivation, soil physicochemical properties, soil bacterial community, sustainable agriculture

1 Introduction

The Asia-Pacific region is among the world's largest producers and consumers of tobacco, with China recognized as one of the leading tobacco-growing countries (Su et al., 2020). Tobacco is a crucial economic crop in our nation, occupying approximately one-third of the global cultivation area (Zou et al., 2018), thereby playing an essential role in national development. It acts as a safeguard for national fiscal revenue while simultaneously promoting economic and social advancement. The use of fertilizers has significantly contributed to global agricultural prosperity (Jez et al., 2016) and has facilitated advancements in the tobacco cultivation sector. However, in their pursuit of higher yields, many tobacco farmers apply large quantities of fertilizers over extended periods. The excessive use of inorganic fertilizers can lead to various issues (Dang et al., 2022), including disruption of soil structure, alterations in soil physicochemical properties, and disturbances in the soil microbial ecological balance (Rahman et al., 2021a). Soil is home to a diverse array of microbial species that perform vital functions (Abed et al., 2013). Growing evidence highlights the critical roles of rhizosphere microorganisms in crop growth and development. Soils with appropriate microbial community structures are essential for robust plant growth and development (Van Bruggen et al., 2019).

In agricultural cultivation, the physicochemical properties of soil and the composition of soil microbial communities significantly influence crop appearance and quality (Zhang et al., 2012). As known, fertilization can alter soil microbial diversity and community composition, which in turn affects soil enzyme activity (Juan et al., 2008). Meanwhile, the investigation of soil microbial structure is vital under different cropping systems. Studies have demonstrated that continuous monoculture can lead to the occurrence of crop rotation obstacles, thereby disrupting the diversity of the soil microbial community (Zhou et al., 2014). Traditional fallow practices may slightly ameliorate degraded soils but could also result in nutrient leaching and the potential dissemination of pathogens within the soil (Ali et al., 2019). Cultivating plant diversity, such as through intercropping, companion planting, and relay cropping, can enhance ecosystem health and promote agricultural sustainability (Ali et al., 2021; Ghani et al., 2022). In China, tobacco cultivation is typically conducted through crop rotation systems. Crop rotation has been shown to enhance bacterial diversity and nitrogen cycling in tobacco soils, while also improving the growth efficiency of tobacco plants (Jin et al., 2024). However, during crop rotation, other crops (such as rice and maize) absorb substantial amounts of nutrients from the soil, leading to a decline in soil fertility (Wang et al., 2024). Therefore, the application of inorganic fertilizers to replenish these depleted nutrients is essential to meet the growth

requirements of tobacco. Nevertheless, excessive use of inorganic fertilizers can reduce crop disease resistance and contribute to ecological degradation. In contrast, organic fertilizers, particularly microbial fertilizers, have been proven to improve soil quality, increase soil organic carbon input, and enhance microbial populations, thereby positively impacting crop production (Sabir et al., 2021). To promote optimal crop growth, it is essential to investigate the effects of fertilizers on crop development and soil microorganisms. Muhammad et al. demonstrated that a blend of 70% compost and 30% chemical fertilizer was more effective than pure chemical fertilizer. Additionally, organic fertilizers can increase the input of soil organic carbon, fostering the proliferation of heterotrophic microorganisms. By combining organic and microbial fertilizers, it is possible to achieve consistently high crop yields while maintaining soil ecological integrity (Hasnain et al., 2020). Sara et al. found that integrating low-concentration chemical fertilizers with high-concentration microbial fertilizers enhances the diversity of eukaryotic organisms in the soil (Sara et al., 2024). Yang et al. highlighted that appropriate cultivation practices and fertilization are crucial for improving soil structure, preserving moisture, and maintaining soil productivity (Yonghui et al., 2022). By exploring the effects of fertilizers on soil and soil microorganisms, valuable insights can be gained to support sustainable agricultural production.

Soil microorganisms enhance plant growth and strengthen plants' resilience against both biotic and abiotic stressors, thereby playing crucial roles in crop protection and soil improvement (Ruan et al., 2020). Beneficial microbial communities can directly reduce disease incidence or lower disease risks by promoting the growth of tobacco plants (Huihui et al., 2021). Dang et al. demonstrated that incorporating manure significantly enhances bacterial diversity in acidic and low-fertility soils (Rahman et al., 2021b). In contrast to conventional fertilizers, microbial organic fertilizer (MOFs) comprise a diverse range of functional probiotics, organic matter, and other beneficial components (Yan et al., 2024). These fertilizers are characterized by their rapid effectiveness and lasting impact. Wu et al. found that microbial formulations in composite microbial fertilizers can stabilize the ecological balance of soil (Xiong et al., 2017). Furthermore, Feng et al. reported that the application of microbial fertilizers significantly alleviates phosphorus limitations in soils used for tobacco cultivation (Feng et al., 2023). Shang et al. investigated the effects of applying *Bacillus subtilis* microbial fertilizer and plant growth-promoting rhizobacteria (PGPR) microbial fertilizer on tobacco plants, revealing that these applications not only alter the soil microbial community structure but also enhance the catalytic conversion efficiency of soil enzymes (Shang et al., 2023). Currently, MOFs are a focal point in the fertilizer industry; however, research on these fertilizers remains in its infancy, and their application at an industrial scale

is limited. Additionally, the standalone use of MOFs can be costly, and their efficacy may be influenced by various environmental and geographical factors, necessitating adjustments in fertilization ratios across different regions.

Therefore, this study aimed to investigate the impact of MOF on tobacco growth in Hunan Province. Experiments were conducted in Changjiang Village, Renyi Town, Guiyang County. The control group consisted of conventional fertilizers containing cake fertilizer, while the experimental group comprised fertilizers containing MOF. The study assessed the effects of MOF on soil physicochemical properties and rhizosphere microbial communities throughout four growth stages of tobacco plants. We hypothesize that (1) the application of MOF could alter the soil microbial diversity at different growth stages of tobacco, (2) MOF improves the physicochemical properties of the soil to enhance soil fertility, and (3) the application of MOF could improve the quality and yield of tobacco leaves.

2 Materials and methods

2.1 Study area and experimental site

The selected experimental site is located in Changjiang Village, Renyi Town, Guiyang County, Chenzhou City, Hunan Province, China (112°23'26"E to 112°55'46"E and latitude 25°37'15"N to 26°13'30"N). Guiyang County has an average annual temperature that ranges from 16°C to 23°C, which is favorable for tobacco cultivation. The selected tobacco field is a tobacco-rice rotation system, and the soil is classified as red soil.

2.2 Soil sampling and experimental design

In this study, two distinct fertilization methods were established, namely, CK and MOF. The CK group served as the control group, receiving only the base fertilizer. In addition to the base fertilizer, the MOF group was designated as the experimental group, supplemented with MOF. The base fertilizer comprised a one-time application of 900 kg ha⁻¹ of specialized tobacco compound fertilizer, 227 kg ha⁻¹ of potassium silicate mineral fertilizer (a slow-release potassium fertilizer), and 136 kg ha⁻¹ of specialized tobacco seedling fertilizer. The MOF applied in the experimental group was 600 kg ha⁻¹, with a 1:1 ratio of Palygorskite to *Bacillus*. Three plots were selected for both the control and experimental groups, with each plot consisting of five rows, each measuring 10 m in length, for a total area of 65 m². A 0.5-meter-wide walkway was incorporated between the plots to facilitate inspection and sampling, and protective measures were implemented on the experimental site. Each plot received base fertilizer before the transplanting period, with fertilization occurring once during the rosette stage, once during the prosperously growing stage, and once during the maturing stage. The timing of fertilization was carefully managed to ensure that the soil moisture levels in each plot were consistent with those observed during previous years' fertilization practices in the local area. The daily management of tobacco is uniformly overseen by the Chenzhou Tobacco Company in accordance with the national standard GB/T 23221-2008 (Chen et al., 2024).

Soil and tobacco sampling were conducted using a five-point sampling method (Hu et al., 2023; Li X. et al., 2024). This approach begins with the selection of the midpoint of the diagonal of the experimental land as the central sampling point, followed by the identification of four equidistant points along the diagonal as additional sampling locations. During four distinct growth stages of the tobacco plants, we selected robust specimens exhibiting uniform growth and collected their rhizosphere soil. The specific procedure is as follows: first, gently uproot the tobacco plants along with their roots, then carefully shake to remove any loose soil from the roots. Next, utilize a sterile brush to collect the root soil, and finally, place the collected soil in sample bags for transportation back to the laboratory, ensuring refrigeration for subsequent testing. We collected a portion of fresh soil, weighed it, and then air-dried it to determine its moisture content. The dried soil was subsequently ground into a fine powder in preparation for the analysis of its physicochemical properties. Another aliquot of fresh soil was taken for microbial analysis. For the sampling of tobacco leaves, we sampled the tobacco leaves 28 days after applying the fertilizer during the maturing stage. The samples were immediately subjected to measurements of relevant physical properties. Afterward, some samples were used for curing, while the remaining samples were used for further analysis of physicochemical properties.

2.3 Physicochemical properties of soil

We utilized the established methodology to examine the soil's physicochemical properties (Wang Z. et al., 2022; Yu X. et al., 2023) as outlined below: Each sample was divided into three parallel portions. The original soil samples were sieved using a 20-mesh sieve (1 mm diameter) to remove stones, plant roots, and residues. The physicochemical properties measured included available potassium (AK), available phosphorus (AP), organic matter (OM), pH, nitrate nitrogen (NO₃⁻-N), and ammonium nitrogen (NH₄⁺-N). Available potassium (AK) was quantified using flame photometry, while available phosphorus (AP) was assessed via the 0.5 mol/L NaHCO₃ method. Organic matter (OM) was quantified using the potassium dichromate volumetric method with external heating. pH was measured with a pH meter (Sartorius Scientific Instruments) at a soil-to-water ratio of 1:2. Nitrate nitrogen (NO₃⁻-N) was determined through the phenol disulfonic acid colorimetric method, and ammonium nitrogen (NH₄⁺-N) was analyzed using the 2 mol/L KCl extraction method for ammonia-nitrogen.

2.4 DNA extraction and PCR amplification

Drawing from prior research (Yu X. et al., 2023), we implemented consistent DNA extraction and PCR amplification methodologies. This process entailed utilizing the DNeasy® PowerSoil® Pro Kit DNA kit (QIAGEN, United States) for extracting genomic DNA of soil microbial communities from 0.5 g soil samples. Subsequently, DNA purity and concentration were determined using a UV spectrophotometer, and the extracted DNA was assessed via 1% agarose gel electrophoresis. To amplify the highly variable regions of bacterial 16S rRNA V3-V4, the primer pairs 338F (5'-ACTCCTACGGGAGCAGCAGC-3') and 806R (5'-

GGACTACHVGGGTWTCTAAT-3') were adopted (Zhang et al., 2022). The amplification protocol consisted of a pre-denaturation step at 95°C for 3 min, followed by denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s. This cycle was repeated 30 times, with a final storage step at 10°C. For the identification of PCR products, three replicate PCR samples were pooled, and the resulting products were analyzed using 2% agarose gel electrophoresis. Finally, the PCR products underwent purification using the AxyPrep DNA Gel Extraction Kit.

2.5 Illumina MiSeq sequencing

Sequencing was performed using the Illumina MiSeq PE300 platform. In this process, one end of the DNA segment is immobilized on a chip, while the other end is hybridized with a complementary buried adapter that is anchored nearby. Subsequent PCR amplification results in the formation of DNA clusters, where the amplified DNA strands are linearized into single strands. Modified DNA polymerase and fluorescently labeled dNTPs are introduced to synthesize one base per cycle. The reaction plate surface is scanned with a laser to read the nucleotide type incorporated during the initial round of template sequencing. Chemical cleavage of the “fluorescent group” and “terminator group” restores the 3' hydroxyl groups, allowing for the addition of the subsequent nucleotide. The fluorescence signals obtained in each cycle are then analyzed to determine the sequence of the template DNA segment.

2.6 Processing of sequencing data

Initially, the raw sequencing data underwent quality control using fastp version 0.20.0 software. Following this, the FLASH software (version 1.2.7) was utilized for sequence assembly. The assembly process comprised several steps: (1) filtering out bases with quality scores below 20 from the ends of the reads using a 50 bp sliding window, (2) merging paired-end reads based on their overlap relationships, requiring a minimum overlap length of 10 bp, (3) permitting a maximum mismatch rate of 0.2 within the overlap region of the merged sequences and discarding those that did not satisfy this criterion, and (4) distinguishing samples using barcodes and primers located at both ends of the sequences, adjusting sequence orientation while allowing zero mismatches for barcodes and a maximum of two mismatches for primers. After completing quality control and assembly, the optimized sequences were denoised using the Deblur plugin in the Qiime2 pipeline with default parameters.

2.7 Analysis of tobacco physical characteristics

Analysis of tobacco physical characteristics was conducted following the national standard GB 2635-1992 (Ling et al., 2020). The appearance quality of tobacco encompasses the sensory and experiential factors through which individuals assess its overall quality. Typically, the evaluation of tobacco grade quality

involves visual inspection, tactile examination, auditory assessment, and olfactory perception. The primary factors influencing tobacco quality include color, maturity, leaf structure, oil content, color intensity, width, and length. The external appearance of tobacco not only reflects its internal quality but also serves as the principal criterion for grading during the procurement process. Generally, high-quality tobacco is characterized by optimal maturity, a loose texture, moderate thickness, golden or orange coloration, adequate oil content, a strong luster, and good elasticity. Tobacco can be classified into upper, middle, and lower leaves, each exhibiting distinct external traits. Leaf length is defined as the straight measurement from the base of the main vein to the leaf tip. Grading standards categorize leaf lengths into five groups: equal to or greater than 45 cm, 40 cm, 35 cm, 30 cm, and 25 cm, with increments of 5 cm. These specifications align with different grade requirements, ensuring that a specific grade meets or exceeds the designated length.

2.8 Data analysis

α -diversity was analyzed using the mothur software platform (v.1.30.2). α -diversity was measured using Hill numbers and Pielou's evenness index. The Shannon index and Simpson index are indicators of community diversity; the ACE index and Chao index are indicators of community richness; and the Coverage index is an indicator of community coverage (Hill, 1973). Statistical analyses and graphical representations, including Venn diagrams, community composition analysis plots, PCoA, RDA, and correlation heatmaps, were performed using R (3.3.1). LEfSe software was used for multilevel species differential analysis. FAPROTAX (version 1.2.1) was employed for differential testing of functional groups. BugBase software was used for BugBase phenotypic prediction. Further comparative analysis of species annotation results was conducted based on the Majorbio Biocloud analysis platform. Variance significance tests for soil physicochemical properties were conducted using SPSS software.

All sequence data were submitted to the Sequence Read Archive (Accession Number: PRJNA1205160) and can be freely accessed through the NCBI website (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1205160>).

3 Results

3.1 The influence of MOF on the physicochemical properties of soil

This study examined the effects of MOF on the physicochemical properties of soil (Table 1). Relative to the control group, the application of MOF had a minimal impact on soil pH, which was consistently maintained at approximately 6.5. However, significant differences ($p < 0.05$) were observed in soil organic matter (OM), available potassium (AK), and available phosphorus (AP) across the four distinct growth stages. In the Experimental group, the AK levels increased during the transplanting stage, peaked at 778.90 mg/kg during the rosette stage, and subsequently declined at the maturation stage. The

TABLE 1 The soil physicochemical properties of the sample groups.

	pH	OM	AK	AP	NO ₃ ⁻ -N	NH ₄ ⁺ -N
T-CK	6.56 ± 0.06abc	13.34 ± 0.13f	122.90 ± 2.03f	9.69 ± 0.59e	3.79 ± 1.13bc	7.88 ± 0.78b
T-MOF	6.56 ± 0.06b	12.23 ± 0.25e	171.94 ± 2.54e	26.46 ± 0.50c	1.36 ± 1.53ab	9.78 ± 7.94b
R-CK	6.65 ± 0.02a	50.12 ± 0.34c	515.46 ± 2.60c	56.83 ± 1.94b	9.45 ± 6.92c	5.60 ± 1.26b
R-MOF	6.53 ± 0.02bc	51.06 ± 0.31a	778.90 ± 19.04a	124.00 ± 9.16a	12.82 ± 3.22a	6.91 ± 3.05b
P-CK	6.62 ± 0.10abc	44.61 ± 1.22f	129.10 ± 8.03f	18.38 ± 0.24d	11.02 ± 0.93a	25.59 ± 1.03a
P-MOF	6.63 ± 0.08ab	43.94 ± 0.60ef	145.55 ± 33.85ef	11.13 ± 1.38e	11.28 ± 6.04a	24.02 ± 2.50a
M-CK	6.55 ± 0.07abc	35.23 ± 0.12b	577.12 ± 6.09b	22.13 ± 2.56cd	3.77 ± 1.24bc	26.55 ± 8.67a
M-MOF	6.53 ± 0.02bc	46.76 ± 0.35d	213.07 ± 6.36d	29.13 ± 1.00c	1.74 ± 0.54c	31.10 ± 2.50a

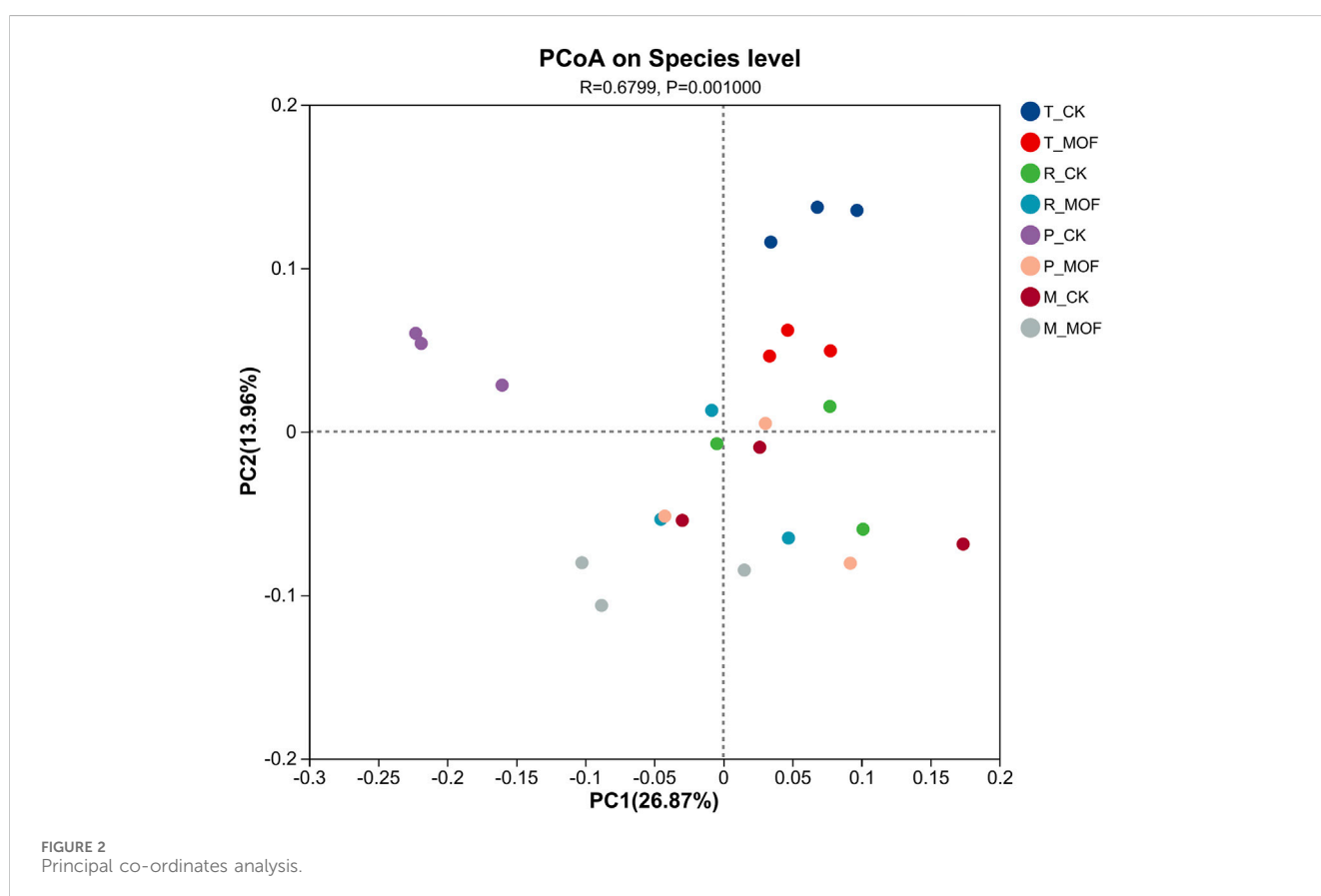
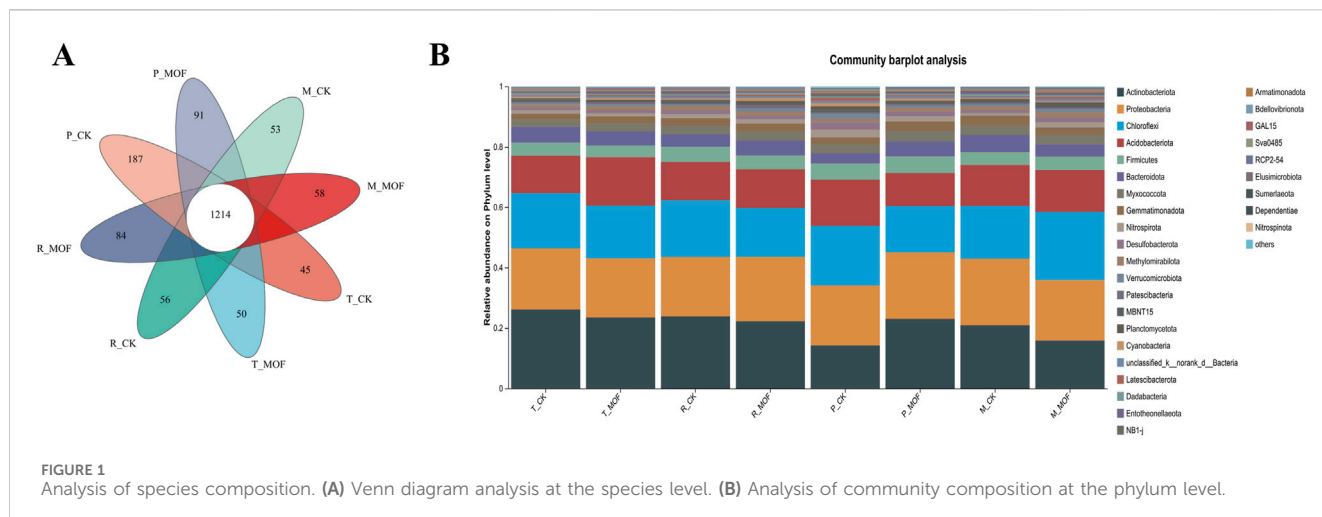
Note: In the same column, the same represents the same period, while different lowercase letters indicate significant differences between the control group and the experimental group ($p < 0.05$). T, transplanting period, R, rosette stage, P, prosperously growing stage, M, maturing stage. CK, control group, MOF, experimental group. pH, pH in soil, OM, organic matter, AK, available potassium in soil, AP, available phosphorus in soil, NO₃⁻-N, nitrate nitrogen in soil, NH₄⁺-N, ammonium nitrogen in soil.

nitrate nitrogen levels decreased during both the transplanting and maturation stages but remained relatively stable during the vigorous growth stage, at approximately 11 mg/kg. Compared to the CK group, the ammonium nitrogen concentration in the Experimental group increased during all three stages.

3.2 The impact of MOF on the microbial composition of rhizospheric soil

To assess the impact of MOF on soil microbial composition, this study conducted α -diversity analysis to evaluate the richness and diversity of microbial communities during different stages: transplanting period, rosette stage, prosperously growing stage, and maturing stage (Supplementary Table S1). The results revealed that the coverage of each sample approached 0.99, indicating sufficient coverage. During the transplanting period and maturing stage, the Shannon index was higher for MOF-treated soil (6.980) compared to CK (7.110) and MOF (7.110) compared to CK (6.803), indicating that MOF promote increased community diversity during these stages. The Simpson index showed lower values for MOF during the transplanting period [MOF (0.002) < CK (0.007)] and maturing stage [MOF (0.004) < CK (0.006)], suggesting that MOF enhance ecosystem diversity in tobacco plant rhizospheric soil. Analysis of Shannon and Simpson indices indicated differences in microbial diversity during the transplanting and maturing stages due to MOF application. Compared to the CK control treatment, the Shannon index slightly increased and the Simpson index slightly decreased during the transplanting and maturing stages following MOF treatment, indicating an increase in the total quantity of microbial organisms in tobacco plant rhizospheric soil. However, we also observed that during the prosperously growing stage, the Shannon index decreased, while the Simpson index increased. The Ace and Chao indices were CK (5589.205) > MOF (5265.152) and CK (5456.055) > MOF (5149.431), respectively, indicating that the diversity of the soil microbial community and bacterial richness declined when plants treated with MOF entered the prosperously growing stage.

The Neutral Community Model (NCM) effectively estimated the primary relationship between the occurrence frequency of operational taxonomic units (OTUs) and changes in their relative abundance (Supplementary Figure S1). The turnover rates of the bacterial community in response to MOF treatment and the Control Check (CK) at four time points were 90.26%, 87.20%, and 92.76%, respectively. The effective population size (N_m) of the bacterial community following MOF treatment ($N_m = 31,035.46$) was greater than that of the CK control ($N_m = 23,814.63$). Both groups exhibited a sequence number of 49,233, with an m value of 0.6304 for MOF treatment and 0.4837 for the CK control. These findings indicate that bacterial species dispersal in the soil significantly increased after MOF treatment compared to the CK control. To identify the specific microbes influenced by MOF, we analyzed the microbial composition in the soil (Figure 1). The results from the Venn diagram indicate that MOF significantly influences the composition of bacteria in the rhizosphere soil of tobacco plants (Figure 1A). At the species level, variations in the number of unique bacteria were observed between the control (CK) and experimental group (MOF) groups across four time points. During the transplanting period, the CK group exhibited 45 unique bacterial genera, whereas the Experimental group displayed 50. In the rosette stage, the counts increased to 56 for CK and 84 for MOF. However, during the prosperously growing stage, the CK group had a notable decline to 187 unique genera, while the Experimental group decreased to 91. In the maturing stage, the numbers were 53 for CK and 58 for MOF. The total number of shared bacteria across all groups was 1,214. An analysis of community composition at the phylum level (Figure 1B) revealed that the predominant bacterial taxa in the soil included *Actinobacteriota*, *Proteobacteria*, *Chloroflexi*, *Acidobacteriota*, *Firmicutes*, *Bacteroidota*, and *Myxococcota*, collectively accounting for over 80% of the total soil bacteria. *Actinobacteriota* emerged as the dominant phylum, comprising approximately 19%–26% of the overall count. *Proteobacteria* was the second most dominant, accounting for about 20%, with specific percentages of 20.26% (T-CK), 19.54% (T-MOF), 19.66% (R-CK), 21.28% (R-MOF), 19.87% (P-CK), 22.06% (P-MOF), 22.03% (M-CK), and 19.96% (M-MOF). Although the proportions of each bacterial taxon differed between the experimental and control groups, these bacterial taxa

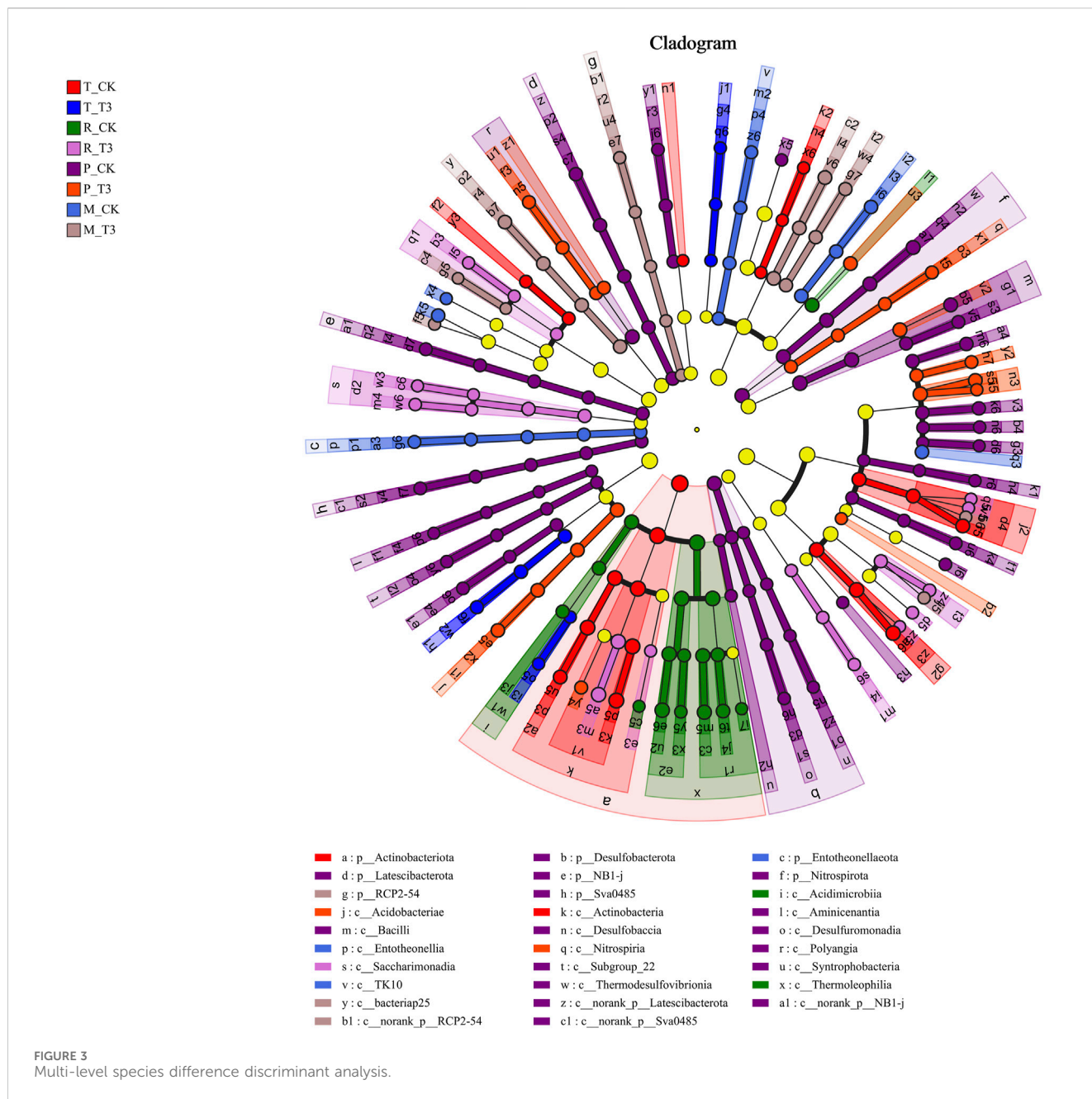


remained dominant in the soil overall, indicating their relatively stable presence in the soil.

Principal Coordinate Analysis (PCoA) at the species level was conducted to examine the differences in soil bacterial community composition between the MOF treatment group and the control group (Figure 2). The results ($R = 0.6799$, $P = 0.0010$) demonstrated significant differences in bacterial communities between the treatment and control groups, particularly during the transplanting period and prosperous growth stage. A distinct separation between the treatment and control groups was

observed at these two stages, indicating that the application of MOF influenced the composition of soil bacterial communities.

Indicative microbes refer to microbial communities that exhibit statistically significant differences. To identify microbial communities with significant features in soil following the application of MOF, we performed LEfSe analysis (Figure 3). Among the bacterial community taxa-level classification units, 189 were identified ($LDA \text{ score} > 3$, $P < 0.05$). The taxa *Blastocatellales*, *Micrococcaceae*, *Nitrosomonadaceae*, and *norank_c_bacteriap25* in the soil treated with MOF displayed significant

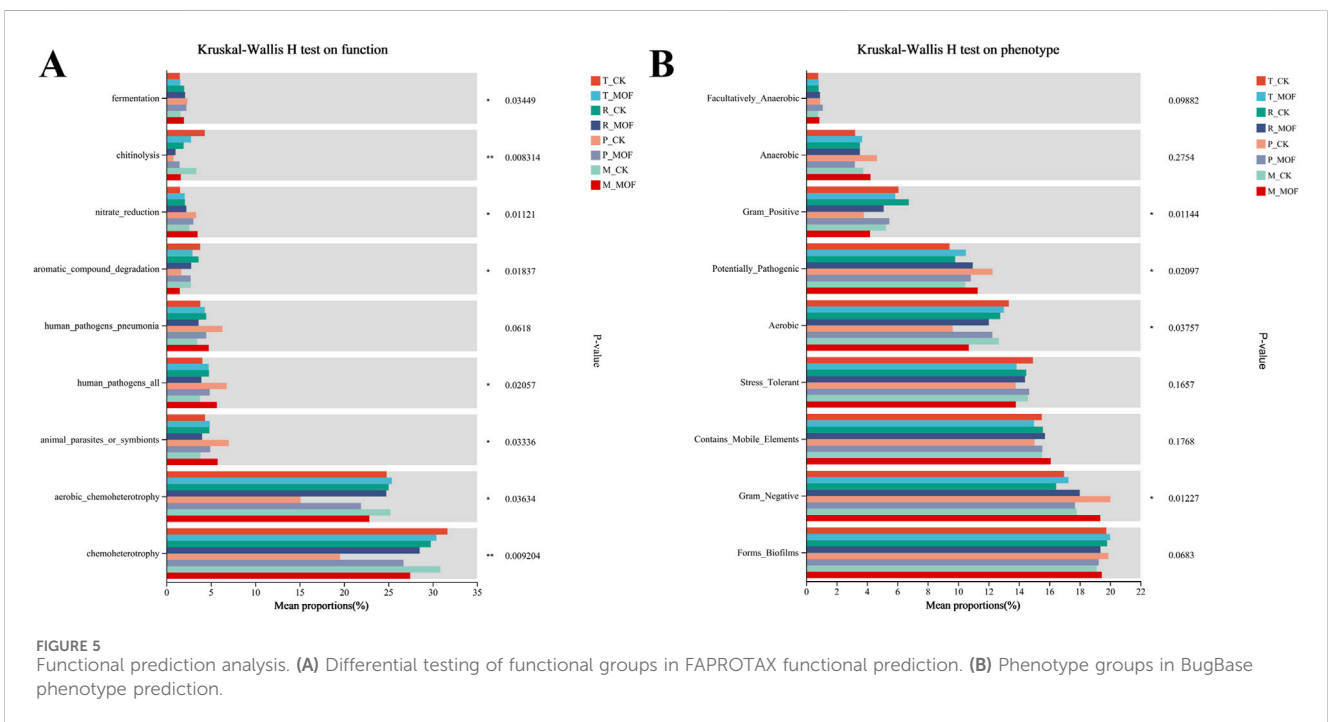
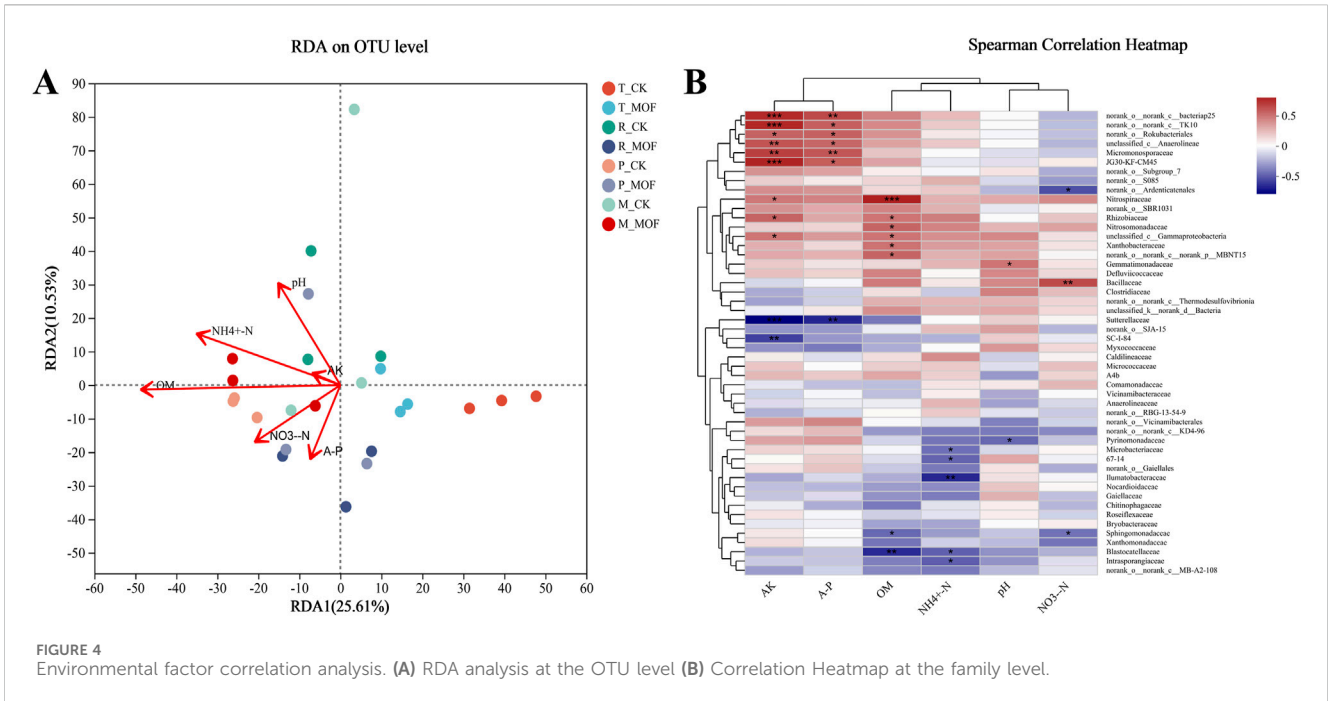


features during the transplanting period, rosette stage, prosperous growth stage, and maturation stage, respectively. In contrast, the soil of the control group exhibited significant features of *Actinobacteriota*, *Thermoleophilia*, *Bacilli*, and *Oxalobacteraceae* across the four periods.

3.3 MOF influence the interactions between environmental factors and microbial community

To elucidate the relationship between microbial communities and environmental factors, a Redundancy Analysis (RDA) was conducted (Figure 4A). RDA1 and RDA2 accounted for 23.75%

and 5.84% of the variance in bacterial community structure, respectively. In comparison to the control (CK), the bacterial communities in the MOF treatment at four different periods were more significantly influenced by available phosphorus (AP), with this regulation being particularly pronounced during the rosette stage. To assess the impact of species abundance, an analysis of the top 50 species at the family classification level, in conjunction with physicochemical properties, was performed (Figure 4B), revealing significant correlations. Notably, norank_o_norank_c_bacteriap25, norank_o_norank_c_TK10, norank_o_Rokubacteriales, unclassified_c_Anaerolineae, Micromonosporaceae, and JG30-KF-CM45 exhibited strong positive correlations with available potassium (AK) and AP, while *Sutterellaceae* demonstrated a significant negative correlation with both AK and



AP. Additionally, *Nitrospiraceae* showed a significant positive correlation with organic matter (OM) and a positive correlation with AK, whereas *Blastocatellaceae* exhibited a significant negative correlation with OM. *Ilumatobacteraceae* displayed a significant negative correlation with ammonium nitrogen (NH₄⁺-N), and *Blastocatellaceae* also showed a negative correlation with both NH₄⁺-N and OM. These findings suggest that MOF play a crucial role in influencing the abundance of bacterial species.

3.4 The impact of MOF on bacterial community function

To further investigate the effects of MOF on the functioning of soil bacterial communities in tobacco inter-roots, we utilized the FAPROTAX database for predictions (Figure 5A). The findings indicated an enhancement in the fermentation and nitrate reduction functions of the rhizosphere bacterial community following the application of MOF during the transplanting period, rosette

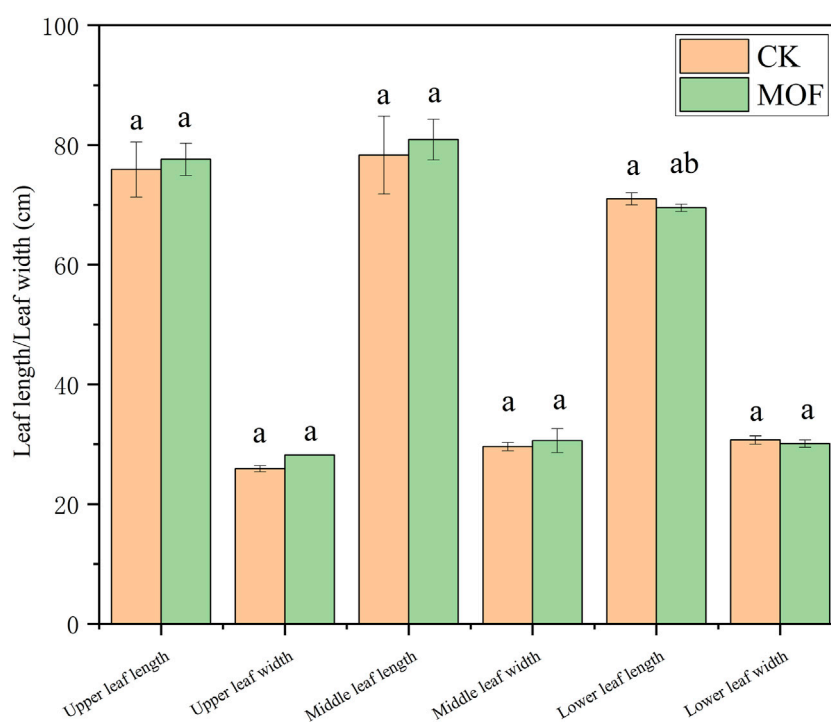


FIGURE 6
Agronomic morphology of tobacco leaves from different positions.

stage, and maturing stage, with a decrease observed during the prosperously growing stage. Conversely, chitinolysis, aromatic compound degradation, and chemoheterotrophy exhibited significant increases during the prosperously growing stage, while experiencing declines during the other three periods. Furthermore, the analysis of the phenotypes of bacterial communities in tobacco rhizosphere soil post-application of MOF, conducted using the BugBase database (Figure 5B), revealed significant differences across four aspects: Gram-Positive, Potentially Pathogenic, Aerobic, and Gram-Negative.

3.5 The impact of MOF on tobacco leaves

The application of MOF was able to cause a more pronounced effect on tobacco leaf traits (Figure 6). Although compared to the CK group, MOF increased the length and width of the upper and middle leaves and reduced the length and width of the lower leaves, statistical analysis revealed that there were no significant differences between the two groups. The application of MOF leads to a 21.93% increase in yield and a 27.38% increase in yield value per unit area while maintaining the average price per plot (Supplementary Table S2). The analysis revealed significant differences between the experimental and control groups in terms of yield and yield value ($p < 0.05$). Furthermore, the appearance quality of cured tobacco leaves (Table 2), revealing an enhancement in leaf color after MOF treatment, with increases of 2.95% for upper leaves, 4.22% for middle leaves, and 3.42% for lower leaves. The total score of leaf quality varies: upper leaves increase by 2.95%, middle leaves by 2.51%, but lower leaves decrease by 1.35%. Oil content in

leaves remains relatively unchanged in upper leaves but decreases in lower and middle leaves. Regarding color intensity, all three leaf segments show improvement after MOF treatment, with the greatest increase observed in lower leaves at 9.76%. Finally, chemical composition analysis of cured tobacco leaves (Supplementary Table S3) reveals an increase in potassium content and a decrease in nicotine and nitrogen content. The reduction of nicotine and total nitrogen content in tobacco is conducive to improving tobacco quality.

4 Discussion

The research suggests that the soil's physicochemical properties underwent changes following the application of MOF. Soil pH remained relatively stable across four distinct stages, aligning with Jiang et al.'s results (Jiang et al., 2021a). When the soil pH is around 6.5, the availability of the nutrients in the soil is relatively high. The root system of tobacco plants can absorb these nutrients more effectively, which thereby promotes the growth of the tobacco plants (Nagao, 1971; Zhao et al., 2024). Nitrogen and potassium are vital elements for plant development (Feng et al., 2018). The slow-release mechanism of convex-concave rods and *Bacillus*'s ability to convert insoluble potassium into soluble forms led to an increase in available potassium content during the transplanting period, rosette stage, and prosperously growing stage compared to the control (El-Hadad et al., 2011). Concurrently, *Bacillus* facilitated potassium utilization in the soil (Abdel-Megeed, 2012), with potassium ions extensively utilized during the maturing stage, resulting in a decline in soil potassium ion concentration. This decline is corroborated by

TABLE 2 Analysis of the post-baking tobacco leaf appearance quality.

Tobacco leaves	Sample	Color	Maturity	Leaf structure	Oil	Color intensity	Total worth
Upper tobacco leaves	CK	23.7 ± 0.6bc	19.8 ± 0.4a	11.8 ± 0.5a	5.0 ± 0.6ab	4.2 ± 0.3ab	74.7 ± 2.7ab
	MOF	24.4 ± 0.2ab	20.4 ± 0.4a	12.3 ± 0.2a	5.0 ± 0.2ab	4.3 ± 0.1ab	76.9 ± 1.0ab
Middle tobacco leaves	CK	23.7 ± 0.3b	19.9 ± 0.1a	11.9 ± 0.5a	6.4 ± 0.2ab	4.3 ± 0.1c	79.6 ± 0.0d
	MOF	24.7 ± 0.2a	20.2 ± 0.1a	12.4 ± 0.4a	6.3 ± 0.2ab	4.6 ± 0.1a	81.6 ± 0.5a
Lower tobacco leaves	CK	23.4 ± 0.6c	19.6 ± 0.4abc	9.7 ± 0.8a	6.7 ± 0.2a	4.1 ± 0.3b	74.2 ± 0.2a
	MOF	24.2 ± 0.2ab	20.1 ± 0.1ab	9.1 ± 1.3a	6.3 ± 0.2a	4.5 ± 0.1ab	73.2 ± 3.2a

Note: In the same column, different lowercase letters indicate significant differences between the control group and the experimental group ($p < 0.05$). CK, control group, MOF, experimental group.

the augmented potassium ion content in the final leaves. Tobacco is a plant that prefers nitrate nitrogen (Duan et al., 2022). When the nitrogen demand of tobacco plants is relatively low, they preferentially utilize nitrate nitrogen. The use of ammonium nitrogen as a nitrogen source may inhibit plant growth, reduce tobacco leaf yield, and impair quality. However, MOF can mitigate the absorption of ammonium nitrogen by plants, ultimately resulting in a decrease in soil nitrate nitrogen content and an increase in ammonium nitrogen content in the treated group.

This study investigates the rhizospheric soil microbial composition of tobacco plants across four distinct growth stages, based on the strong correlation between plant growth and microbial ecology characteristics. α -Diversity analysis revealed that MOF significantly influenced the bacterial diversity in the rhizosphere soil of tobacco plants, particularly during the prosperously growing stage. The increased nutrient demand by the plants intensified competition among microbial communities. Additionally, the application of MOF accelerated the succession process of bacterial communities, such as *Bacillus*, further exacerbating inter-bacterial competition, which led to a decline in diversity and richness. Previous studies have demonstrated that the application of *Bacillus* can reduce soil species diversity (Li et al., 2022), and that plants, when faced with increased nutrient demands, compete with microorganisms for limited nutrient resources through root exudates and other mechanisms (Ruan et al., 2024). These findings are consistent with our observations. NCM analysis reveals that the R^2 value of the Experimental group exceeds that of the control (CK) group, with both the m value and Nm value also elevated in comparison to the control (CK) group. A higher R^2 value indicates a closer alignment with a neutral model, suggesting that stochastic processes have a more significant influence on community assembly. Furthermore, an increased m value reflects a reduction in constraints on species dispersal, while a higher Nm value denotes a more uniform distribution of species across communities (Zhou and Ning, 2017). This implies that a greater number of species can migrate among different communities, thereby enhancing species diversity and richness within these communities. Consequently, the application of MOF appears to enhance microbial dispersal, corroborating the results of Liu et al.'s study on soil microbial communities (Liu X. et al., 2022).

Analyzing the rhizospheric soil microbial communities of tobacco plants at the phylum level reveals that Actinobacteriota and Proteobacteria are the first and second dominant phyla, respectively, comprising 39%–46% of the total soil bacterial

population. This finding is consistent with previous reports (Verzeaux et al., 2016). Actinobacteriota serve as crucial decomposers in the soil, playing a significant role in nutrient transformation and utilization (Jenkins et al., 2009). In contrast, Proteobacteria display remarkable environmental adaptability and possess metabolic degradation functions (Emenike et al., 2023; Gong et al., 2023; Liu B. et al., 2022). Chloroflexi is the third dominant phylum, primarily involved in the decomposition of organic matter and carbon cycling in soil. They are capable of degrading complex organic compounds, thereby promoting the cycling of soil organic matter and the maintenance of soil fertility (Xian et al., 2020). Acidobacteriota, as the fourth dominant phylum, can solubilize minerals in the soil, releasing phosphorus and other trace elements essential for plant growth (Yu Y. et al., 2023). Furthermore, Principal Coordinate Analysis (PCoA) indicates that the application of MOF influences the bacterial communities in the soil, corroborating findings from previous studies (Jenkins et al., 2009; Sun et al., 2015; Wang B. et al., 2022).

LEfSe analysis revealed the microbial communities associated with specific functions and exhibiting significant features following the application of MOF (Du et al., 2022). For example, during the transplanting period after the application of these fertilizers, *Blastocatellales* displayed significant features. As a key player in the network of organic and natural farming systems (Matteoli et al., 2022), *Blastocatellales* not only decompose organic matter in the soil but also participate in the nitrogen cycling process, indicating a notable association between the reduction of nitrate nitrogen during the transplanting period and *Blastocatellales*. Additionally, *Micrococcaceae* exhibited significant features during the rosette stage; according to Lu et al., *Micrococcaceae*, recognized as a salt-tolerant bacterium, can promote plant growth (Lu et al., 2023). Furthermore, *Nitrosomonadaceae*, which are ammonia-oxidizing bacteria, facilitate soil nitrification by converting nitrites to nitrates (Cheng et al., 2017; Chun et al., 2021).

The shape, yield, and quality of tobacco leaves are the most direct and effective indicators for evaluating the efficacy of MOF. In addition to improving crop yield, MOF can enhance fertilizer utilization efficiency and reduce environmental pollution associated with chemical fertilizers (Liu et al., 2015; Shao et al., 2025). In this study, the application of MOF significantly increased both the yield and value of tobacco, while also improving the quality of the leaves in comparison to the control group (CK). This finding is consistent with previous research (Zhang et al., 2013). Moreover, nicotine is one of the main sources of nitrogen in tobacco, and the

reduction in nitrogen content is usually associated with a reduction in nicotine content. Therefore, the reduction in nicotine and total nitrogen content in tobacco leaves could contribute to lowering the toxicity of tobacco and improving the quality of the leaves (Jiang Y. et al., 2021; Li Z. J. et al., 2024). The improvement in the quality of tobacco leaves could further enhance their economic value.

The bacterial community in this study underwent further analysis for functional predictions using FAPROTAX and phenotypic predictions using BugBase. In the FAPROTAX functional prediction, significant differences were observed in the chitinolysis and chemoheterotrophy functions of soil microorganisms. With the exception of the prosperously growing stage, during which the control group surpassed the experimental group, the experimental group exhibited higher levels than the control group in the other three periods. This suggests that organic fertilizers can significantly enhance the chitinolysis and chemoheterotrophy functions of soil microbial communities in the tobacco rhizosphere. BugBase phenotype prediction primarily indicated that, apart from the prosperously growing stage, the Gram-positive and aerobic phenotype types of microorganisms in the experimental group were higher than those in the control group, while the potentially pathogenic and Gram-negative phenotype types of microorganisms in the experimental group were lower than those in the control group. However, these hypotheses lacked experimental validation, resulting in disparities between predicted outcomes and actual observations (Du et al., 2022; Tao et al., 2020; Wang T. et al., 2022). Therefore, subsequent experiments should aim to validate the impact of changes in microbial communities on microbial community functions. It is worth noting that this study was conducted on the same land within the same year, and did not explore the effects of MOF in tobacco planting soils across different regions, which may limit its geographical applicability. In addition to altering soil microbial communities and improving tobacco yield and quality through MOF, interspecific planting of different plants has been shown to modify the composition of rhizospheric soil microbes (Zhou et al., 2023). Additionally, altering planting systems is another effective approach (Liu et al., 2020). Furthermore, balancing microbial community structure by reducing nitrogen fertilizer application can promote tobacco plant growth (Shen et al., 2022). Hence, various methods can be employed in subsequent studies to investigate the effects on soil microbes. The most effective approach to studying the effects of MOF on soil microbes in tobacco rhizospheric soil would involve conducting experiments in different regions, with different tobacco plants, and across different years. However, given the complexity of microbial interactions with soil physicochemical properties and plants, there remains substantial research potential in using MOF to improve soil quality and enhance plant growth (Gu et al., 2021; Li et al., 2021).

5 Conclusion

This study investigated the effects of MOF on microbial community structure, soil physicochemical properties, and

tobacco leaf quality in rhizosphere soils. The results indicated that MOF can enhance levels of soil organic matter, available phosphorus, and ammonium nitrogen, while also increasing microbial diversity, particularly by promoting beneficial bacterial groups such as Actinobacteria and Proteobacteria. These changes in microbial communities contribute to improved soil fertility, which subsequently enhances the quality and yield of tobacco leaves. These findings may provide valuable insights for further research into the role of MOFs in promoting crop growth and increasing agricultural yields. Future studies should focus on long-term field trials to assess the persistence of these effects, explore microbial functional traits through metagenomic analysis, and investigate plant-microbe interactions to optimize the application of microbial fertilizers within agricultural systems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/sra/PRJNA1205160>.

Author contributions

ShW: Conceptualization, Formal Analysis, Methodology, Supervision, Writing–review & editing. BZ: Conceptualization, Formal Analysis, Investigation, Methodology, Writing–review & editing. SM: Data curation, Investigation, Methodology, Writing–review & editing. JHa: Investigation, Methodology, Writing–review & editing. LZ: Investigation, Methodology, Writing–review & editing. CG: Methodology, Supervision, Writing–review & editing. JHo: Investigation, Methodology, Writing–review & editing. HD: Data curation, Writing–review & editing. YZ: Investigation, Writing–review & editing. YW: Data curation, Methodology, Writing–review & editing. WW: Data curation, Methodology, Writing–original draft. JS: Writing–original draft. SX: Writing–original draft. JY: Conceptualization, Funding acquisition, Supervision, Writing–review & editing. GS: Funding acquisition, Resources, Supervision, Writing–review & editing.

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Conflict of interest

Authors ShW, BZ, SM, JHa, LZ, CG, JHo, HD, and YZ were employed by Inner Mongolia Kunming Cigarette Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2025.1555622/full#supplementary-material>

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