MICROBIAL DIVERSITY IN RHIZOSPHERE OF TOBACCO INFECTED BY MELOIDOGYNE SPP UNDER DIFFERENT CONTROL MEASURES

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ABSTRACT

This research explores the microbial diversity in tobacco root rhizosphere of root-knot nematode (Meloidogyne spp.)-infected tobacco under different biocontrol measures in Renhe District, Panzhihua. Fifty tobacco root rhizosphere soil samples collected in 2017 from tobacco fields were analyzed for bacterial and fungal communities. The results are as follows: (1) Crotalaria juncea and Avermectin treatments yielded the highest bacterial and fungal OTU numbers, respectively. Among other treatments (Paecilomyces lilacinus, Heterorhabditis bacteriophora), bacterial OTU numbers were higher than the control (CK) except for H. bacteriophora. Fungal OTU numbers in the other two treatments (H. bacteriophora and Avermectin) were lower than CK. C. juncea and CK showed the highest and lowest bacterial α-diversity indices (Simpson and ACE), respectively, while P. lilacinus and C. juncea exhibited the highest and lowest fungal α-diversity indices. (2) Proteobacteria, the dominant bacterial phylum, showed no significant differences in relative abundance among PP (0.3906), EPNs (0.3828), and CK (0.3862) ($p>0.05$), but these values were significantly higher than CJ (0.3431) and AV (0.3081) ($p<0.05$). The dominant fungal phylum Ascomycota maintained relative abundances exceeding 0.7785 across all treatments, with no inter-treatment differences ($p>0.05$). (3) Comparative analysis identified 10 dominant bacterial genera and 7 fungal genera in CJ, 10 bacterial and 3 fungal in PP, 8 bacterial and 5 fungal in EPNs, 11 bacterial and 4 fungal in AV, and 9 bacterial and 6 fungal in CK. C. juncea significantly enhanced soil bacterial and fungal diversity compared to other biocontrol methods.

KEYWORDS

Root knot nematode; Microbial communities; Tobacco; Sunn hemp

1. INTRODUCTION

Tobacco (Nicotiana tabacum L.) is a globally pivotal cash crop extensively grown in tropical and subtropical zones including major producers China, the United States, and Turkey. Nevertheless, its yield and quality are severely restricted by root-knot nematodes (Meloidogyne spp.)-obligate endoparasites that invade root tissues, induce gall formation, disrupt nutrient/water uptake, and impair plant growth. As a major soil-borne pathogen in tobacco agriculture, Meloidogyne spp. cause 15%-60% yield loss[1], threatening the global tobacco industry's economic sustainability.

Currently, chemical nematicides dominate Meloidogyne spp. management. Yet long-term over-reliance on synthetic nematicides raises critical environmental concerns: they accumulate in soil to reduce microbial diversity and fertility, and pose toxic risks to non-target organisms (e.g.,

beneficial insects, soil fauna) and human health via the food chain[2]. Thus, developing eco-friendly, sustainable biocontrol alternatives has become an urgent priority in plant nematology.
Emerging biocontrol strategies focus on diverse agents (BCAs) and materials, categorized as: (1) leguminous cover crops (e.g., Crotalaria juncea L., Crotalaria pallida Aiton) inhibiting nematode reproduction via allelopathy or as trap crops; (2) entomopathogenic fungi (e.g., Paecilomyces lilacinus (Thom) Samson) parasitizing nematode eggs/juveniles; (3) microbial metabolites (e.g., avermectin) disrupting nematode nervous systems; (4) entomopathogenic nematodes (e.g., Steinernema spp., Heterorhabditis spp.) releasing symbiotic bacteria to kill nematodes[3]. These alternatives have gained attention for their environmental safety and ability to preserve soil health while suppressing nematodes.

Notably, Meloidogyne spp. Pathogenesis is exacerbated by rhizospheric microbial interactions-either promoting disease via secondary pathogen colonization[4] or enhancing plant protection via microbial antagonism[5-6]. The rhizosphere, first defined by Hiltner[7], is a dynamic microbial hotspot where plant-microbe interactions shape ecosystem functioning[8], with fungal communities key to nutrient cycling and pathogen suppression [9]. Clarifying these microbial dynamics is critical for optimizing biocontrol strategies.

This study aimed to: (1) quantify Meloidogyne spp. infestation severity under different biocontrol measures; (2) characterize interactions between Meloidogyne spp. and rhizospheric microbiota; (3) profile microbial community composition, abundance, and diversity; (4) identify correlations between treatment-induced microbial shifts and nematode suppression efficacy.

2. MATERIALS AND METHODS

2.1. Field experiment

Seeds of C. juncea and entomopathogenic nematodes (H. bacteriophora) were provided by Yunnan Green Leaf Bio-control Technology Co., Ltd., Yuxi, Yunnan, China. Seedlings of Yunyan 87 tobacco were provided by the Panzhihua Branch of Sichuan Tobacco Corporation, Panzhihua, Sichuan, China. P. lilacinus powder was provided by Zhongxiang Biological Engineering Co., Ltd., Hubei, China, and 0.5% avermectin granules were provided by Weifang Chinese Agriculture Chemical Industry Co., Ltd., Weifang, Shandong, China.

The experiment was conducted in a randomized complete block design in tobacco fields from May to October 2017 in Panzhihua, Sichuan, China. When the tobacco plants were transplanted, five treatments (labeled A, B, C, D, and E) were established:

A: 8250 seeds of C. juncea (CJ) per hectare were sown between two adjacent tobacco plants within the same row;
B: 1000 IJS/plant of H. bacteriophora (EPNs) was applied around the roots;
C: 10 billion cfu/g Paecilomyces lilacinus (PP) powder (2 g/plant) was applied around the roots;
D: No treatment was used as the control (CK);
E: 0.5% avermectin (AV) granules (3 g/plant) were applied around the roots.
Each plot contained 2 rows of tobacco plants, with 30 plants per row, a row spacing of 1.2 m, and a plant spacing of 0.5 m. Twelve plots were randomly arranged, with three replicates per treatment.

2.2. Soil sample collection

In October 2017, rhizosphere soil samples were collected from a field (26°15□N, 101°52□E) cultivated with tobacco (Yunyan 87) for eight consecutive years in Renhe District, Panzhihua City, Sichuan Province-the major tobacco-producing area of Panzhihua. The field was naturally infested with root-knot nematodes (Meloidogyne spp.) in aggregated (spotty/patchy) patterns. The soil was classified as red soil (sandy loam) with moderate fertility. Fifty plots were sampled in the field, corresponding to the rhizosphere soils of treatments A to E. Rhizosphere soil samples from individual plants were placed in separate sterile bags as individual samples and immediately stored in foam boxes packed with dry ice.

2.3. Sequencing method

2.3.1. Extraction of genome DNA

Total genomic DNA from rhizosphere soil samples was extracted using the CTAB/SDS protocol. DNA concentration and purity were assessed on 1% agarose gels. Based on the quantified concentrations, DNA samples were diluted to 1 ng/μL with sterile water.

2.3.2. Amplicon Generation

Primer: 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R, ITS1: ITS1F-ITS2. The 16S/18S rRNA genes were amplified using barcoded specific primers. All PCR reactions were performed in 30μL volumes containing 15μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2μM forward and reverse primers, and ~10 ng of template DNA. Thermal cycling conditions included: an initial denaturation at 98°C for 1min; 30 cycles of denaturation at 98°C for 10 sec, annealing at 50°C for 30 sec, and elongation at 72°C for 60 sec; with a final extension at 72°C for 5 min.

2.3.3. PCR Products quantification and qualification

Mix the same volume of 1X loading buffer (containing SYBR® Green) with PCR products and perform electrophoresis on a 2% agarose gel for detection. Samples showing a bright main band between 400 and 450 bp were selected for further experiments.

2.3.4. PCR Products Mixing and Purification

The PCR products were mixed at equimolar ratios. Subsequently, the mixed PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific).

2.3.5. Library preparation and sequencing

Sequencing libraries were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) according to the manufacturer's instructions, with incorporated index codes. Library quality was evaluated using the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific) and the Agilent 2100 Bioanalyzer system. Finally, libraries were sequenced on an Illumina MiSeq platform, generating 250/300 bp paired-end reads.

2.4. Data analysis

Paired-end reads were merged using FLASH v1.2.11[10], a high-throughput tool designed for assembling overlapping reads (Read 1 and Read 2). Reads were demultiplexed to individual samples based on unique barcodes. Sequence analysis was conducted through the QIIME pipeline

(v1.9.1; Quantitative Insights Into Microbial Ecology)[11], supplemented with custom Perl scripts for α-diversity (intra-sample) and β-diversity (inter-sample) analyses.

First, raw reads were filtered using QIIME's quality control parameters. Operational Taxonomic Units (OTUs) were clustered de novo via the pick_de_novo_otus.py script at a 97% sequence similarity threshold. Sequences sharing ≥97% similarity were grouped into the same OTUs. Representative OTU sequences were taxonomically classified with the RDP Classifier v2.12[12] against the SILVA 132 SSU rRNA reference database.

α-diversity metrics (Chao1 richness, observed OTUs, and Shannon index) were calculated from rarefied OTU tables to normalize sequencing depth heterogeneity. Rarefaction curves were generated for these metrics. β-diversity was assessed using both weighted and unweighted UniFrac distances. Unweighted UniFrac distances were utilized for Principal Coordinate Analysis (PCoA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering.

PCoA transforms distance matrices into orthogonal axes, where the first principal coordinate explains the maximum variance, followed by the second coordinate, and so on. UPGMA clustering, an average-linkage hierarchical method, was applied to build phylogenetic trees from unweighted UniFrac distance matrices.

3. RESULTS

3.1. Analysis of OTU abundance and α diversity index of bacteria

Raw sequencing data were generated from rhizosphere soil samples of tobacco subjected to five treatments (Table 1). Treatments A to E yielded 92,976, 92,983, 75,440, 84,312, and 89,362 raw sequences, respectively; after quality filtering, the number of effective sequences was 89,528, 90,205, 73,368, 82,045, and 86,766 for each treatment. At a 97% sequence similarity threshold, bacterial sequences were clustered into 3,733-4,445 operational taxonomic units (OTUs) across the five treatments.

Analysis of α-diversity indices (Simpson and ACE) revealed that bacterial diversity in four biocontrol treatments was significantly higher than that in the blank control (CK, Treatment D) ($p<0.05$). Specifically, Treatment B (PP) exhibited the highest α-diversity, followed by Treatment A (Crotalaria juncea, CJ), Treatment E (Avermectin, AV), and Treatment C (Heterorhabditis bacteriophora, EPNs). The coverage value of all samples exceeded 0.970, indicating that the sequencing libraries effectively captured the majority of bacterial taxa and accurately reflected the bacterial community structure in the rhizosphere soil.

Table 1. Bacterial OTU Abundance and α-Diversity Indices

Sample name	Raw date	Qualified data	OUT number	α diversity index				
				Shannon index	Chao1 index	Simpon	ACE	coverage
A	92976	89528	4445	9.811	0.997	4471.886	4594.144	0.972
B	92983	90205	4305	9.883	0.997	4727.603	4820.222	0.970
C	75440	73368	3733	9.568	0.996	4018.765	4059.634	0.976
D	84312	82045	3781	9.496	0.996	3732.264	3847.984	0.977
E	89362	86766	4191	9.739	0.996	4431.691	4584.770	0.972

Sample A to E correspond to CJ, PP, EPNs, CK, AV respectively.

3.2. Analysis of OTU abundance and α diversity index of fungi

Raw sequencing reads for fungal communities in tobacco rhizosphere soil are presented in Table 2. Treatments A to E generated 86,826, 82,921, 92,356, 96,027, and 90,226 raw sequences, respectively; after removing low-quality sequences, the number of effective sequences was 83,729, 80,087, 88,382, 92,864, and 88,244. At a 97% sequence similarity threshold, fungal sequences were clustered into 978-1,109 OTUs across the five treatments.

α-diversity analysis showed that Treatment A (CJ) had the highest Shannon (6.247) and Chao1 (0.966) indices, while Treatment B (PP) exhibited the highest Simpson (1,307.639) and ACE (1,118.728) indices, collectively indicating that these two treatments promoted fungal diversity more effectively than others. The coverage value of all treatments exceeded 0.997, demonstrating that the sequencing libraries comprehensively captured the fungal community diversity and accurately represented the fungal community structure.

Table 2. OTU abundance and α diversity index of fungi

Sample name	Raw date	Qualified data	OUT number	α diversity index				
				Shannon index	Chao1 index	Simpon	ACE	coverage
A	86826	83729	978	6.247	0.966	954.751	973.977	0.998
B	82921	80087	1003	6.055	0.961	1307.639	1118.728	0.997
C	92356	88382	1024	6.041	0.958	1000.777	1015.545	0.998
D	96027	92864	1090	6.039	0.954	1061.889	1082.332	0.998
E	90226	88244	1109	6.016	0.951	1094.813	1114.013	0.997

Sample A to E correspond to CJ, PP, EPNs, CK, AV respectively.

3.3. Analysis on bacterial community composition

3.3.1. Phylum-Level Composition

At the phylum level (Figure 1), the bacterial communities in rhizosphere soil across all five treatments were dominated by Proteobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, Acidobacteria, Bacteroidetes, Thaumarchaeota, Chloroflexi, Planctomycetes, Verrucomicrobia, and unclassified groups. No significant differences were observed in the relative abundances of Actinobacteria and Firmicutes among treatments ($p>0.05$).

Proteobacteria was the most dominant bacterial phylum: its relative abundances in PP (0.3906), EPNs (0.3828), and CK (0.3862) were not significantly different ($p>0.05$), but all were significantly higher than those in CJ (0.3431) and AV (0.3081) ($p<0.05$). For Gemmatimonadetes, CK had the highest relative abundance (0.0993), followed by AV (0.0825), EPNs (0.0813), and CJ/PP (both 0.0746); the relative abundance of Gemmatimonadetes in CK was significantly higher than that in other treatments ($p<0.05$).

In Acidobacteria, EPNs (0.1112) and AV (0.1016) had the highest relative abundances (no significant difference between them, $p>0.05$), followed by CJ (0.0983) and PP (0.0946); the relative abundances of Acidobacteria in these four treatments were significantly higher than that in CK (0.0653) ($p<0.05$). Bacteroidetes had the highest relative abundance in CJ (0.0537), followed by PP (0.0414), AV (0.0391), and CK (0.0334) (no significant differences among these three treatments, $p>0.05$), while EPNs had the lowest relative abundance (0.0229), which was significantly lower than that in other treatments ($p<0.01$).
For Planctomycetes, the relative abundances in CJ (0.0245), EPNs (0.0229), and AV (0.0226) were not significantly different ($p>0.05$), followed by PP (0.0209) and CK (0.0139); the relative abundance of Planctomycetes in CK was significantly lower than that in other treatments

($p<0.05$). Verrucomicrobia had the highest relative abundance in CJ (0.0194), followed by PP (0.0179), EPNs (0.0177), and AV (0.0163) (no significant differences among these three treatments, $p>0.05$), while CK had the lowest relative abundance (0.0117), which was significantly lower than that in other treatments ($p<0.01$).

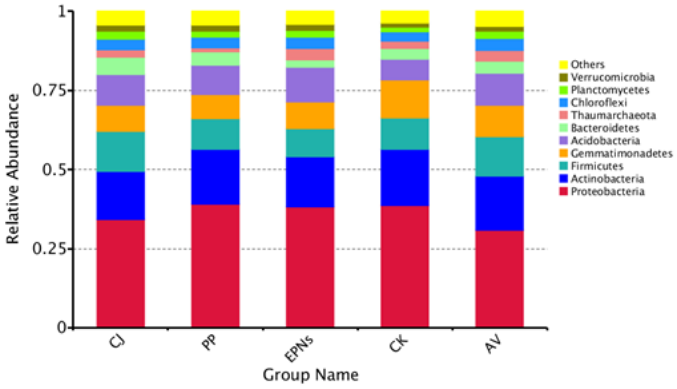

Figure 1. Bacterial Abundance and Composition from the Perspective of Phylum

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.3.2. Class-Level Composition

At the class level (Figure 2), the bacterial communities in rhizosphere soil across the five treatments were primarily composed of Alphaproteobacteria, Betaproteobacteria, unidentified Gemmatimonadetes, unidentified Actinobacteria, Gammaproteobacteria, Thermoleophilia, Clostridia, Deltaproteobacteria, SCG, Bacilli, and unclassified groups. No significant differences were observed in the relative abundances of most bacterial classes among treatments, except for Alphaproteobacteria, unidentified Gemmatimonadetes, and unidentified Actinobacteria.

Alphaproteobacteria was the dominant bacterial class: CK had the highest relative abundance (0.1779), which was not significantly different from that in PP (0.1746) ($p>0.05$), followed by EPNs (0.1638), CJ (0.1444), and AV (0.1356). For unidentified Gemmatimonadetes, CK had the highest relative abundance (0.1143), followed by AV (0.0950), while EPNs (0.0794), CJ (0.0774), and PP (0.0708) had lower relative abundances (no significant differences among these three treatments, $p>0.05$).

Unidentified Actinobacteria had the highest relative abundance in CK (0.0960), followed by AV (0.0931), PP (0.0868), and CJ (0.0795), while EPNs had the lowest relative abundance (0.0777); no significant differences were observed among treatments ($p>0.05$).

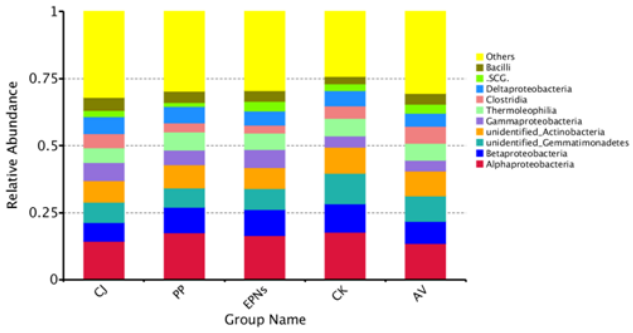

Figure 2. Bacterial Abundance and Composition from the Perspective of Class

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.3.3. Genus-Level Composition

At the genus level (Figure 3), the bacterial communities in rhizosphere soil across the five treatments were mainly distributed among Sphingomonas, Gemmatimonas, Acinetobacter, Pseudarthrobacter, Solirubrobacter, Lachnospiraceae NK3A20 group, Haliangium, Bryobacter, Lactobacillus, Staphylococcus, Mizugakiibacter, Pseudomonas, Succinivibrio, Bacillus, Megasphaera, Acidibacter, Olsenella, Methyloversatilis, Bradyrhizobium, Roseiflexus, Rikenellaceae RC9 gut group, RB41, unidentified Nitrospiraceae, Pseudolabrys, Dialister, Ruminococcus 1, Acidobacterium, Delftia, H16, Steroidobacter, and unclassified groups. Marked differences were observed solely with respect to the relative abundances of Gemmatimonas, Pseudarthrobacter, Staphylococcus, Pseudomonas, and Acidibacter among treatments ($p<0.05$).

Gemmatimonas had the highest relative abundance in CK (0.0380), followed by CJ (0.0252), EPNs (0.0237), and PP (0.0220) (no significant differences among these three treatments, $p>0.05$), while AV had the lowest relative abundance (0.0279). Staphylococcus had the highest relative abundance in CJ (0.0114), followed by PP (0.0035), while AV, EPNs, and CK had the lowest relative abundances (no significant differences among them, $p>0.05$).

Pseudomonas had the highest relative abundance in EPNs (0.0092), followed by CJ (0.0024), AV (0.0018), and PP (0.0010) (no significant differences among these three treatments, $p>0.05$), while CK had the lowest relative abundance (0.0007). Acidibacter had the highest relative abundance in PP (0.0114), which was not significantly different from that in CK (0.0104) and EPNs (0.0103) ($p>0.05$), followed by CJ (0.0090), while AV had the lowest relative abundance (0.0062).

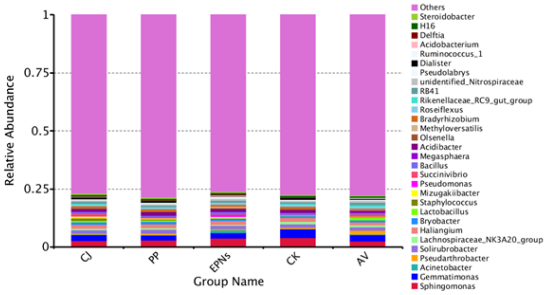

Figure 3. Bacterial Abundance and Composition from the Perspective of Genus

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.3.4. Species-Level Composition

At the species level (Figure 4), the bacterial communities in rhizosphere soil across the five treatments were mainly composed of Acinetobacter baumannii, Pseudarthrobacter oxydans, Lactobacillus iners, Staphylococcus aureus, Sphingomonas paucimobilis, Bradyrhizobium elkanii, Lactobacillus jensenii, Nitrospira japonica, Delftia tsuruhatensis, Megasphaera sp. UPII 199-6, and unclassified groups. Significant variations were detected exclusively in the relative abundances of Pseudarthrobacter oxydans and Staphylococcus aureus among treatments ($p<0.05$).

Pseudarthrobacter oxydans had the highest relative abundance in AV (0.01755), which was significantly higher than that in the other four treatments ($p<0.05$); no significant differences were observed among the other four treatments ($p>0.05$). Staphylococcus aureus had the highest relative abundance in CJ (0.01139), followed by PP, while CK had the lowest relative abundance; no significant differences were observed between CJ, EPNs, and AV ($p>0.05$).

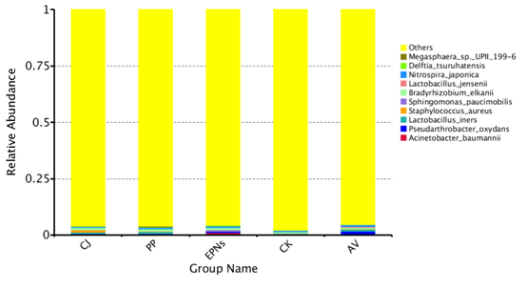

Figure 4. Bacterial Abundance and Composition from the Perspective of Species

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per

plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.4. Analysis on fungal community composition

3.4.1. Phylum-Level Composition

At the phylum level (Figure 5), the fungal communities in rhizosphere soil across the five treatments were mainly distributed among Ascomycota, Mucoromycota, Basidiomycota, Chytridiomycota, Glomeromycota, Neocallimastigomycota, incertae sedis fungi, and unclassified groups. Significant differences were only observed in the relative abundance of Basidiomycota among treatments ($p<0.05$).

Ascomycota served as the dominant fungal phylum, exhibiting a relative abundance exceeding 0.7785 in all treatments (no significant differences among treatments, $p>0.05$). Basidiomycota had the highest relative abundance in CK (0.1256), followed by CJ, PP, and AV (no significant differences among these three treatments, $p>0.05$), while EPNs had the lowest relative abundance.

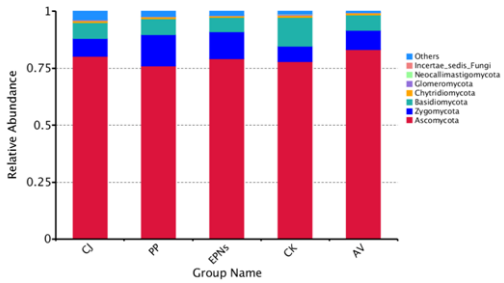

Figure 5. Fungal Abundance and Composition from the Perspective of Phylum

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.4.2. Class-Level Composition

At the class level (Figure 6), the fungal communities in rhizosphere soil across the five treatments were primarily composed of Sordariomycetes, incertae sedis Mucoromycota, Dothideomycetes, Eurotiomycetes, Agaricomycetes, Tremellomycetes, Pezizomycetes, Leotiomycetes, Chytridiomycetes, incertae sedis Ascomycota, and unclassified groups. Noticeable differences were recorded exclusively for the relative abundances of Agaricomycetes and Leotiomycetes among treatments ($p<0.05$).

Agaricomycetes had the highest relative abundance in CK (0.0638), while CJ had the lowest relative abundance; no significant differences were observed between CJ and the other three treatments (PP, EPNs, AV) ($p>0.05$). Leotiomycetes had the highest relative abundance in CJ

(0.03), followed by CK, PP, and AV (no significant differences among these three treatments, $p>0.05$), while EPNs had the lowest relative abundance.

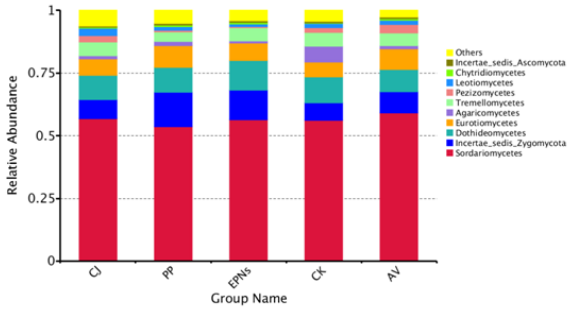

Figure 6. Fungal Abundance and Composition from the Perspective of Class

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.4.3. Genus-Level Composition

At the genus level (Figure 7), the fungal communities in rhizosphere soil across the five treatments were mainly distributed among Gibberella, Fusarium, Aspergillus, Chaetomium, Phoma, Cercophora, Rhizopus, Thielaviopsis, Myceliophthora, Cephalotrichum, Trichocladium, Latorua, Gibellulopsis, Postia, Scytalidium, Pithoascus, Zopfiella, Acrostalagmus, Stephanonectria, Chrysosporium, Lophiostoma, Arthrographis, Thielavia, Mortierella, Fusicolla, Arthrinium, Kernia, Leucoagaricus, Remersonia, Ambispora, and unclassified groups. Distinct disparities were identified only in terms of the relative abundances of Gibberella and Rhizopus among treatments ($p<0.05$).

Gibberella had the highest relative abundance in AV (0.1462), followed by CK, CJ, and EPNs (no significant differences among these three treatments, $p>0.05$), while PP had the lowest relative abundance. Rhizopus had the highest relative abundance in EPNs (0.0290), followed by CJ (0.0224), while AV, CK, and PP had the lowest relative abundances (no significant differences among them, $p>0.05$).

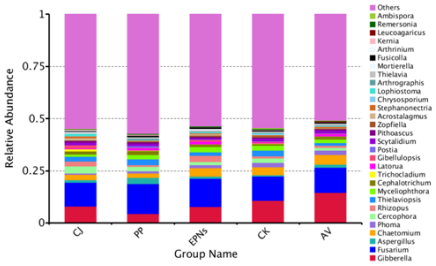

Figure 7. Fungal Abundance and Composition from the Perspective of Genus

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.4.4. Species-Level Composition

At the species level (Figure 8), the fungal communities in rhizosphere soil across the five treatments were mainly composed of Gibberella intricans, Fusarium oxysporum, Aspergillus quadrilineatus, Phoma herbarum, Chaetomium subspirilliferum, Rhizopus arrhizus, Thielaviopsis basicola, Myceliophthora verrucosa, Cephalotrichum microsporum, Cercophora areolata, and unclassified groups. Significant variations were detected exclusively in the relative abundances of Gibberella intricans, Rhizopus arrhizus, and Cercophora areolata among treatments ($p < 0.05$).

Gibberella intricans had the highest relative abundance in AV (0.1443), followed by CK, CJ, and EPNs (no significant differences among these three treatments, $p > 0.05$), while PP had the lowest relative abundance. Rhizopus arrhizus had the highest relative abundance in EPNs (0.0290), followed by CJ (0.0224), while AV, CK, and PP had the lowest relative abundances (no significant differences among them, $p > 0.05$). Cercophora areolata had the highest relative abundance in CJ (0.0190), followed by CK, EPNs, and PP (no significant differences among these three treatments, $p > 0.05$), while AV had the lowest relative abundance.

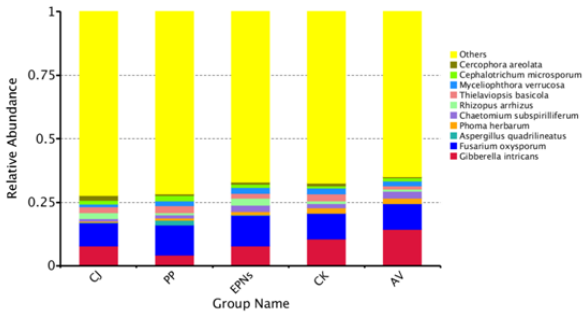

Figure 8. Fungal Abundance and Composition from the Perspective of Species

Note: Different lowercase letters indicate significant differences at the 0.05 level among treatments (Duncan's multiple range test). CJ=Crotalaria juncea (8250 seeds•ha^{-1}, sown between adjacent tobacco plants in the same row); EPNs = Heterorhabditis bacteriophora (1000 IJS per plant, applied around tobacco roots); PP = Paecilomyces lilacinus powder (10^9 cfu•g^{-1}, 2 g per plant, applied around tobacco roots); CK = blank control (no treatment); AV = 0.5% avermectin granules (3 g per plant, applied around tobacco roots). Each treatment had 3 biological replicates, and the data presented are the mean±standard deviation (SD).

3.5. Identification of Dominant Microbial Taxa Across Treatments

By selecting treatments with significant differences in microbial diversity, the dominant bacterial and fungal taxa were identified as follows:

Dominant bacterial taxa: CJ treatment contained 10 dominant taxa (Proteobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, unidentified Actinobacteria, Staphylococcus, Pseudomonas, Acidibacter, Staphylococcus aureus); PP treatment contained 10 dominant taxa (Proteobacteria, Acidobacteria, Planctomycetes, Verrucomicrobia, Alphaproteobacteria, unidentified Actinobacteria, Acidibacter, Staphylococcus, Pseudomonas, Staphylococcus aureus); EPNs treatment contained 8 dominant taxa (Proteobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia, Alphaproteobacteria, Pseudomonas, Acidibacter); AV treatment contained 11 dominant taxa (Acidobacteria, Planctomycetes, Bacteroidetes, Gemmatimonadetes, Verrucomicrobia, unidentified Actinobacteria, unidentified Gemmatimonadetes, Pseudarthrobacter, Gemmatimonas, Pseudomonas, Pseudarthrobacter oxydans); CK treatment contained 9 dominant taxa (Bacteroidetes, Proteobacteria, Gemmatimonadetes, Planctomycetes, Alphaproteobacteria, unidentified Gemmatimonadetes, unidentified Actinobacteria, Gemmatimonas, Acidibacter).

Dominant fungal taxa: CJ treatment contained 7 dominant taxa (Basidiomycota, Leotiomycetes, Gibberella, Rhizopus, Cercophora areolata, Gibberella intricans, Rhizopus arrhizus); PP treatment contained 3 dominant taxa (Basidiomycota, Leotiomycetes, Cercophora areolata); EPNs treatment contained 5 dominant taxa (Gibberella, Rhizopus, Rhizopus arrhizus, Gibberella intricans, Cercophora areolata); AV treatment contained 4 dominant taxa (Basidiomycota, Leotiomycetes, Gibberella, Gibberella intricans); CK treatment contained 6 dominant taxa (Basidiomycota, Agaricomycetes, Leotiomycetes, Gibberella, Gibberella intricans, Cercophora areolata).

Compared with CK, AV, CJ, and PP treatments increased soil bacterial diversity, while CJ treatment significantly enhanced soil fungal diversity ($p < 0.05$).

4. DISCUSSION

The bacterial operational taxonomic unit (OTU) number and α-diversity indices (Simpson index, ACE index) in the rhizosphere soil of the four biocontrol treatment groups (Crotalaria juncea, CJ; Paecilomyces lilacinus, PP; Heterorhabditis bacteriophora, EPNs; Avermectin, AV) were all higher than those in the blank control group (CK). Previous studies have indicated that soil microbial diversity is closely associated with disease suppression effects: an increase in soil microbial diversity can reduce the incidence and severity of soil-borne diseases[13]. The results of this study not only support this viewpoint but also further suggest that it is the soil microbial community structure (rather than total microbial abundance) that determines the occurrence and severity of root-knot nematode disease in tobacco, as even with similar total microbial biomass, differences in community composition among treatments led to distinct disease suppression outcomes.

Current research[14] has proposed that Bacteroidetes is widely recognized as a dominant bacterial phylum across diverse crop species, soil types, and geographical regions. However, data from the present study demonstrated that the relative abundance of Bacteroidetes in the rhizosphere soil across the five treatments (CJ, PP, EPNs, AV, CK) only ranged from 2.29% to 5.37%—a value significantly lower than that reported in other cropland soil ecosystems. This notable imbalance may be related to the unique soil properties of tobacco-growing areas in Panzhihua (e.g., soil pH, nutrient content) or the specific root exudate profiles of tobacco (Nicotiana tabacum L.), which could selectively inhibit the proliferation of Bacteroidetes taxa. Thus, the interaction mechanism between Bacteroidetes and tobacco plants, as well as its potential impact on root-knot nematode suppression, warrants further in-depth investigation.

Analysis of the fungal community composition showed that Ascomycota (relative abundance: 76.04%–83.13%), Basidiomycota (7.63%–17.32%), and Mucoromycota (6.20%–12.56%) were the dominant fungal phyla in the tobacco rhizosphere soil. These fungal phyla are key components of soil ecosystems, as they maintain microbial ecological balance through decomposition of soil organic matter and plant root exudates, thereby regulating nutrient cycling and soil fertility[15]. Notably, among the three dominant fungal phyla, only Mucoromycota exhibited significant differences in relative abundance among treatments, while Ascomycota and Basidiomycota showed relatively stable distribution patterns. Additionally, the relative abundance of Basidiomycota in the CK group was higher than that in the four biocontrol treatment groups—a finding consistent with the results reported by Chen et al. [16], who observed a positive correlation between elevated Basidiomycota abundance and increased root-knot nematode infestation. This suggests that the proportional dynamics of Ascomycota, Basidiomycota, and Zygomycota may be closely correlated with the occurrence and development of root-knot nematode disease in tobacco, and further studies are needed to clarify the specific regulatory roles of these fungal phyla in nematode-host plant interactions.

Comparative analysis of microbial populations across the five treatments revealed that the CJ treatment group contained 10 dominant bacterial taxa and 7 dominant fungal taxa—more than the other treatment groups—indicating that C. juncea has a more significant ability to enhance soil microbial diversity. This impact can be ascribed to the function of C. juncea as a green manure crop: during its growth and decomposition, it releases large amounts of organic matter and nutrients (e.g., nitrogen, phosphorus, potassium) into the soil, providing a rich carbon and energy source for soil microorganisms, thereby promoting the proliferation of diverse microbial taxa. Support for this explanation comes from previous work on the soil health benefits of C. juncea, which have confirmed its ability to improve soil microbial activity and diversity[17-19]. Furthermore, existing research has highlighted C. juncea as a key resource for nematode suppression, and this function is likely mediated by its promotion of natural enemies of nematodes, including fungal egg parasites (e.g., Pochonia chlamydosporia), nematode-trapping fungi (e.g., Arthrobotrys oligospora), and plant growth-promoting rhizobacteria (e.g., Bacillus subtilis)[20]. Aligned with the aforementioned observations, the outcomes of this research demonstrated that at the tobacco harvest stage, the CJ treatment exhibited superior control efficacy against root-knot nematodes (41.9%) compared to the EPNs treatment (39.3%) and PP treatment (39.8%)[3], further confirming the potential of C. juncea as an efficient and sustainable biocontrol measure for tobacco root-knot nematodes.

Combined with a comparative analysis of analogous studies, the superior nematicidal efficacy of Crotalaria juncea is attributed to the synergistic regulation of multiple pathways rather than a single mechanism, which differentiates it distinctly from other biocontrol measures in three core aspects: first, nutrients released during its decomposition facilitate the proliferation of beneficial microorganisms such as Paecilomyces lilacinus (PP) constructing a stable "nutrient supply-beneficial microbe proliferation-nematode suppression" cascade that overcomes the limitations of single biocontrol agents (e.g., PP and entomopathogenic nematodes (EPNs) constrained by soil conditions and predation pressure); second, its secreted allelochemicals (including flavonoids and alkaloids) exert a dual effect by directly inhibiting nematode egg hatching and infection, while indirectly promoting the growth of nematode antagonists, thus avoiding the drawbacks of avermectin (AV)—which induces nematode resistance and disrupts soil microecology—and the transient microecological impacts of rapeseed meal-derived allelochemicals; third, its decomposition elevates soil organic matter content, enhances the stability of soil microbial communities, and establishes a "microecological barrier" against nematode infection, while simultaneously optimizing soil conditions to strengthen tobacco’s intrinsic nematode resistance, ultimately achieving a "soil-microorganism-plant" synergistic optimization that prevents

nematode disease rebound—a unique advantage unattainable by single biocontrol treatments like PP and EPNs.

This study comprehensively analyzed the composition and diversity characteristics of rhizosphere microbial communities in tobacco fields under different biocontrol strategies. In light of comparisons with analogous biocontrol research, it deepened the understanding of the mechanism by which C. juncea controls tobacco root-knot nematodes and laid a theoretical foundation for clarifying the microbial regulatory mechanism of root-knot nematode control. However, this study still has limitations that need to be addressed in future research: for example, it only focused on the microbial community characteristics at a single growth stage of tobacco, and the spatiotemporal dynamics of microbial populations (e.g., dynamic changes across different tobacco growth stages or soil depths) and their specific association with the infection dynamics of root-knot nematodes remain unclear. Meanwhile, in-depth analysis of the specific types and action concentrations of C. juncea allelochemicals, as well as their interaction mechanisms with beneficial microorganisms, has not been conducted. For instance, whether the allelochemicals of C. juncea have a similar mode of action to the volatile substances from cruciferous green manure requires further verification[21]. Therefore, future studies should integrate multi-time-point sampling and multi-dimensional detection technologies (e.g., metagenomics, metatranscriptomics, metabolomics) to systematically explore the spatiotemporal dynamics of rhizosphere microorganisms and the mechanism of action of C. juncea allelochemicals. In addition, drawing on the research idea of inducing biocontrol fungus activity with exogenous substances[22], screening core microbial taxa related to root-knot nematode control to explore efficient biocontrol resources, and constructing combined microbial regulation technologies by integrating multiple biocontrol factors (e.g., combining C. juncea with beneficial microorganisms) to optimize control schemes are expected to provide new technical solutions and theoretical support for the green and sustainable control of tobacco root-knot nematode diseases.

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