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Exploring the Impact of Tea (*Camellia sinensis* (L.) O. Ktze.)/*Trachelospermum jasminoides* (Lindl.) Lem. Intercropping on Soil Health and Microbial Communities

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Abstract: Intercropping, a well-established agroecological technique designed to bolster ecological stability, has been shown to have a significant impact on soil health. However, the specific effects of tea/*Trachelospermum jasminoides* intercropping on the physicochemical properties and functional microbial community structure in practical cultivation have not been thoroughly investigated. In this study, we utilized high-throughput sequencing technology on the 16S/ITS rDNA genes to assess the impact of tea intercropping with *T. jasminoides* on the composition, diversity, and potential functions of the soil microbial community in tea gardens. The results indicated that the tea/*T. jasminoides* intercropping system significantly increased pH levels, soil organic matter, available nitrogen, phosphorus, potassium, and enzyme activity, ultimately augmenting soil nutrient levels. Furthermore, an in-depth analysis of the bacterial co-occurrence network and topological structure portrayed a more intricate and interconnected soil bacterial community in tea gardens. Remarkably, the abundance of beneficial genera, including *Burkholderia*, *Mesorhizobium*, *Penicillium*, and *Trichoderma*, underwent a substantial increase, whereas the relative abundance of pathogenic fungi such as *Aspergillus*, *Fusarium*, and *Curvularia* experienced a marked decline. Functional predictions also indicated a notable enhancement in the abundance of microorganisms associated with nitrogen and carbon cycling processes. In summary, the intercropping of tea and *T. jasminoides* holds the potential to enrich soil nutrient content, reshape the microbial community structure, bolster the abundance of functional microorganisms, and mitigate the prevalence of pathogenic fungi. Consequently, this intercropping system offers a promising solution for sustainable tea garden management, overcoming the limitations of traditional cultivation methods and providing valuable insights for sustainable agriculture practices.

Keywords: intercropping; *Trachelospermum jasminoides*; physicochemical properties; microbial community structure; functional prediction

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1. Introduction

The tea plant (*Camellia sinensis* (L.) O. Ktze.), globally revered as a beloved beverage [1], is cultivated extensively in tropical and subtropical regions [2], with China boasting a staggering 3.17 million hectares dedicated to tea gardens in 2020 [3]. However, in the context of intensified agriculture, tea gardens are predominantly cultivated by using monoculture practices [4]. This prolonged monoculture has led to a reduction in the resilience of tea gardens to natural disasters, affecting factors such as water availability, temperature regulation, and biodiversity, ultimately leading to a decline in productivity [4]. The soil microbial

community plays a fundamental role in soil biology, sustaining soil functions and influencing soil productivity and crop yields [5]. Extensive studies have revealed that the widespread monoculture of teas disrupts the soil microbial community's structure, leading to an increase in pathogenic microorganisms and a decrease in microbial diversity [6,7]. Microorganisms play a crucial role in stabilizing and decomposing organic matter, thereby affecting soil nutrient turnover and enzyme activity. Prolonged monoculture of teas has been proven to adversely impact soil bacterial diversity [4]. Li et al. [8] highlighted that long-term cultivation for 10 and 20 years significantly altered the soil bacterial community, resulting in a decrease in the relative abundance of several beneficial genera, including *Pseudomonas*, *Rhodanobacter*, *Bradyrhizobium*, *Mycobacterium*, and *Sphingomonas*. This poses significant challenges for the sustainable development of tea crops under monoculture practices.

Intercropping is a sustainable agricultural practice involving the simultaneous cultivation of two or more crops [9]. Numerous researchers have demonstrated that intercropping plays a crucial role in promoting sustainable agriculture by influencing soil microbial communities to enhance microbial diversity, carbon assimilation, nitrogen input, nutrient cycling, and pest reduction, ultimately resulting in increased crop yields and optimized quality [10,11]. Research specifically focused on the intercropping of teas has found that different intercropping patterns have a significant impact on soil ecology. For example, intercropping rubber trees in tea gardens could reduce soil water loss, while intercropping with chestnuts and fruit trees (such as loquats, raspberries, and citrus) can lower environmental and soil temperatures, ultimately increasing air and soil humidity within tea plantations [12]. Other woody plants, such as persimmon, when intercropped with teas, not only protect teas from summer glare stress but also improve soil nutrient availability, soil enzyme activity, and the quantity and quality of the tea produced [13,14]. Moreover, the diversity of soil bacteria in the rhizosphere plays a pivotal role in these processes [15,16]. For instance, intercropping soybeans with teas increased the relative abundance of beneficial bacteria such as Acidobacteriaceae, Burkholderaceae, Rhodanobacteraceae, and Sphingomonadaceae, which are known as organic matter decomposers or Plant Growth-Promoting Rhizobacteria (PGPR) [17]. Huang et al. [18] found that soybean–tea intercropping significantly increased the absolute abundance of Bacillus, a type of PGPR associated with promoting crop production [19]. These findings indicate that the choice of plant species and the intercropping patterns have a significant influence on tea soil properties and microbial communities. The complex and dynamic interactions between plants and microbes have thus become a crucial area of agricultural research [20]. However, in mountainous tea plantations with steep terrain, there may be limited options for alternative intercrops. Common intercrop choices, such as legumes and fruit trees, may hinder tea ventilation and pose challenges for cultivation management, which can result in higher costs [21].

Trachelospermum jasminoides, commonly known as star jasmine, is an evergreen woody vine that exhibits tolerance to both cold and heat. It has undemanding soil requirements, rapid growth, strong stress resistance, and a small footprint and provides extensive coverage, making it a popular choice for ecological slope protection. The combination of tea and *T. jasminoides* intercropping has proven to be particularly effective for slope protection in tea gardens, especially those cultivated in terraced fields (personal observation, unpublished data). However, there is currently a lack of research on the impact of tea and *T. jasminoides* intercropping on the soil microbial community structure in tea gardens. Therefore, this study aimed to utilize high-throughput sequencing technology on the 16S/ITS rDNA to investigate the impacts of *T. jasminoides* intercropping on the diversity, structure, and function of the soil microbial community in tea gardens. The primary objectives of this research are as follows: (1) explore the influence of tea and *T. jasminoides* intercropping on soil properties; (2) compare the responses of bacterial and fungal communities, as well as key genera, between the intercropping model and the tea cultivation-only model; and (3) further explore the interaction relationship between the key bacteria and plants and the changes in soil microecology in the tea garden under the *T. jasminoides* intercropping

model and provide a more comprehensive theoretical basis for the promotion of this intercropping model.

2. Materials and Methods

2.1. Overview of Study Area

The experimental site was situated at National Soil and Water Conservation Park of Wuyi University in Wuyishan City, Fujian Province (117.99° E, 27.72° N), at an altitude of 239 m (Figure S1). This region experiences a subtropical monsoon climate with abundant rainfall, and the soil is classified as red acidic soil. The teas used in the experiment were five-year-old Rougui cultivars of tea trees, and the tea garden covered a total area of 12 hectares and was laid out on a terrace with a row spacing of 1.5 m. Every October, a total of 700 kg/ha of compound fertilizer is applied, with a composition of N:P:K = 21:8:16. Two treatments were established: intercropping (TI) and monoculture (TM). Each treatment consisted of four randomly selected plots, each measuring 10 m × 10 m. *T. jasminoides* was planted on the terraces and slopes of the intercropping plots in March 2022, with a minimum distance of 0.3 m from the teas and a planting spacing of 0.18 m × 0.18 m. The cultivation management practices were consistent across different tea gardens.

2.2. Soil Sample Collection

The soil sampling method was based on the study by Zhong et al. [22]. In September 2023, soil samples were collected from the intercropping (TI) area, which was located at the midpoint between the tea trees and *T. jasminoides*, at a depth of 3–13 cm, referred to as intercropping soil. The sampling locations and depths in the monoculture (TM) area were consistent with those in the intercropping area. Each treatment consisted of four plots, and 15 samples were randomly collected from each plot, resulting in a total of four replicates. After collection, the soil samples were preserved in ice bags and transported to the laboratory. In the laboratory, a 100-mesh sieve was employed to remove gravel and visible impurities from the samples. The soil samples were then stored separately in freezers at temperatures of −80 °C and 4 °C for subsequent analysis, which included soil DNA extraction, determination of soil enzyme activity, and measurement of soil nutrient content.

2.3. Determination of Soil Physicochemical Properties and Enzyme Activities

Available nitrogen (AN) was determined by using the alkaline diffusion method, available phosphorus (AP) by the 0.5 mol·L^{-1} sodium bicarbonate leaching method, available potassium (AK) by NH_4OAc leaching flame spectrophotometry. The organic matter content in the soil was measured by using the potassium dichromate titration method [23], and pH was determined by the potential method.

Soil catalase activity (CAT) was determined by the pyrogallol colorimetric method, urease (UE) by the sodium hypochlorite colorimetric method, and polyphenol oxidase (PPO) by the pyrogallol colorimetric method. Acid protease (ACPT) was determined by the colorimetric method with ninhydrin. Soil cellulase activity (CE) was determined by using the anthrone colorimetric method, while soil sucrose activity (IE) was determined by using the 3,5-dinitrosalicylic acid method [23,24].

2.4. Soil Total DNA Extraction

Soil total DNA was extracted by using the BioFast Soil Genomic DNA Extraction Kit (BioFlux, Hangzhou, China). The purity of the extracted DNA was assessed through 1% agarose gel electrophoresis. Subsequently, the DNA concentration was determined by using a NanoDrop2000C Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Only DNA samples with satisfactory quality were employed for high-throughput sequencing analysis of the microbial community.

2.5. High-Throughput Sequencing Analysis of 16S/ITS rDNA

The 16S rDNA and ITS rDNA of soil samples were amplified. See Table S1 for details on primers and thermal cycling procedures. PCR instrument: ABI GeneAmp® Type 9700 (Perkin Elmer, Waltham, MA, USA); Then, the soil microorganisms were sequenced by the Illumina Hiseq sequencing method. Sequencing data were processed on the Qiime platform (http://qiime.org/scripts/assign_taxonomy.html (accessed on 4 June 2024)) to remove low-quality sequences with an average quality score less than 20 ($Q < 20$) and sequences shorter than 100 base pair [25]. By using Uparse to cluster all the Effective Tags of all samples, OTUs were clustered at 97% sequence consistency by default [26,27]. The OTUs sequences were further annotated with the blast method in Qiime and the Unit (v7.2) database to obtain the microbial abundance at different taxonomic levels.

2.6. Data Analysis

Significant analysis was conducted by using IBM SPSS software (version 26; New York, NY, USA), employing ANOVA with the LSD test ($p < 0.05$), and the boxplots were computed by using the "ggplot" package in R software (version 4.3.1; Fort Worth, TX, USA). After normalizing the soil microbial community data, α-diversity and PCoA analyses were performed by using the Bray–Curtis algorithm. Diversity index plots were generated by using GraphPad Prism (version 9.5; San Diego, CA, USA). The co-occurrence network was computed by using the "igraph" package in R software (version 4.3.1; Fort Worth, TX, USA) with Spearman correlation, requiring a correlation coefficient greater than 0.7 and a significant level of $p < 0.05$. Subsequently, Gephi (version 0.9.7; Paris, France) and Cytoscape (version 3.9.1; San Diego, CA, USA) were utilized to visualize the co-occurrence network. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was used to estimate the relative abundance of species, with a logarithmic LDA score threshold of 3.5 [28]. LEfSe analysis was performed to identify significant differences from phylum to genus levels among the three treatment groups and to determine the characteristics most likely to explain the differences among the categories [29]. The vegan software package (version 2.5.6) was used to rank microbial and soil properties by redundancy analysis (RDA) [30]. Random forest analysis was conducted with the "RandomForest" package to identify the proportion of microorganisms. Functional predictions for bacterial and fungal communities in the soil microbiota were performed based on the FAPROTAX and FUNGuild databases, respectively. The data were analyzed by using IBM SPSS Amos (version 28) software to construct a Structural Equation Model (SEM) in order to examine the significance of the relationships between different variables under intercropping.

3. Results

3.1. Impacts of Intercropping on Soil Physicochemical Properties and Enzyme Activities

The intercropping of tea with *T. jasminoides* significantly altered the soil nutrient status. As depicted in Figure 1, the integration with *T. jasminoides* in the intercropping system led to a marked increase in the levels of pH, AN, AP, AK, and SOM beneath the tea plants compared with their monoculture counterparts ($p < 0.05$). Furthermore, the intercropping approach was also observed to significantly boost various enzyme activities, including CE, ACPT, POD, and IE, compared with monoculture ($p < 0.05$). However, the activities of UE and PPO were notably lower in the intercropping system. These findings clearly indicate that the intercropping of teas with *T. jasminoides* significantly affects the soil environment, enhancing the nutrient availability and augmenting diverse enzyme activities.

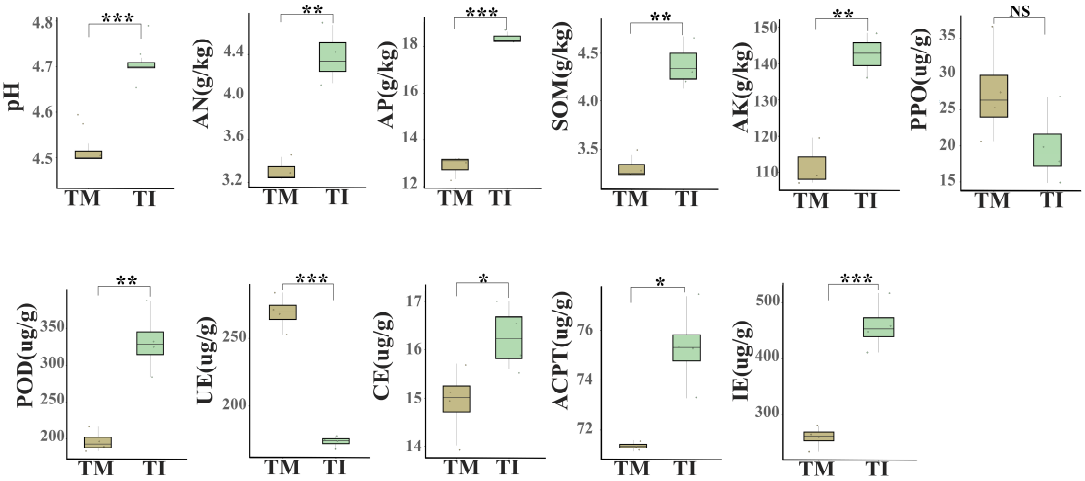

Figure 1. Comparison of soil physicochemical properties and enzyme activity between intercropping (TI) and monoculture (TM). AN: available nitrogen; AP: available phosphorus; AK: available potassium; SOM: organic matter; UE: urease; PPO: polyphenol oxidase; ACPT: acid phosphatase; CE: cellulase; POD: peroxidase; IE: sucrose. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, NS: no significant difference).

3.2. Effects of Intercropping on α-Diversity and β-Diversity of Soil Microorganisms

The intercropping of tea with *T. jasminoides* profoundly influenced the rhizosphere microbial community's diversity. As depicted in Figure 2A,B, the boxplots reveal significant increases in both the Chao1 and Shannon diversity indices (Shannon and Chao1) for the bacterial community when tea was intercropped with *T. jasminoides* ($p < 0.05$). In contrast, the fungal community exhibited a marked increase in diversity indices. A Principal Coordinates Analysis (PCoA) conducted at the OTU level further explored the similarities and dissimilarities in microbial community structure composition under the different treatments. Figure 2C,D displays a distinct separation between the microbial communities of TM and TI. In conclusion, the intercropping of tea with *T. jasminoides* exerts a substantial impact on soil microbial diversity and community structure.

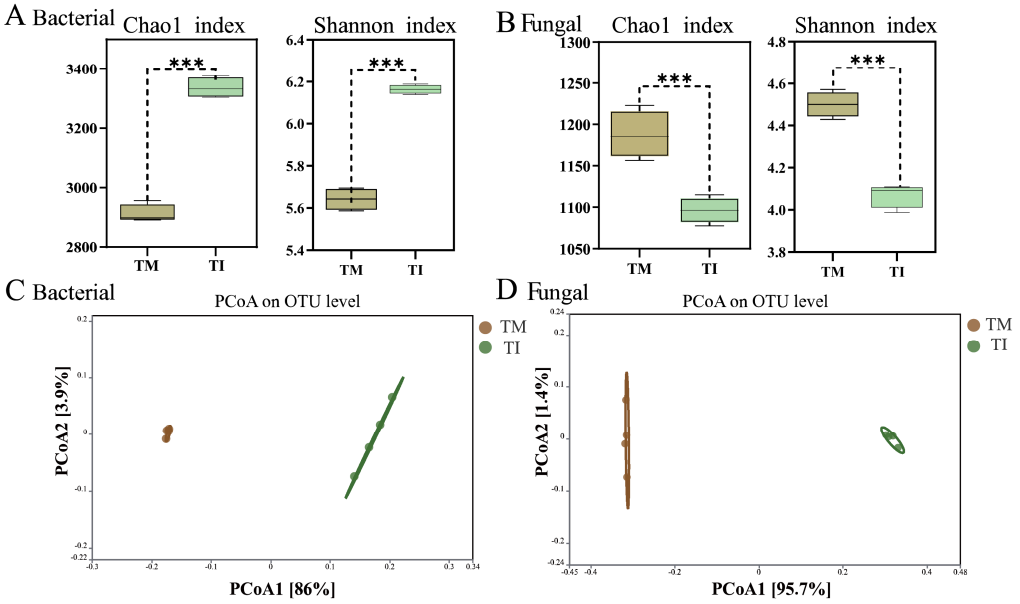

Figure 2. Comparison of soil microbial α-diversity and β-diversity between intercropping (TI) and monoculture (TM). (**A**) Bacterial α-diversity; (**B**) Fungal α-diversity; (**C**) Bacterial β-diversity: PCoA; (**D**) Fungal β-diversity: PCoA. In this context, the notation *** denotes a significance level of $p < 0.001$.

3.3. Impact of Intercropping on Soil Microbial Co-Occurrence Networks

Given the intricate interconnectedness of soil microbial turnover, we conducted a genus-level co-occurrence network analysis to delve deeper into how microbial interactions were affected by tea/*T. jasminoides* intercropping (Table S2). The results show that the bacterial networks in the monoculture treatment comprised 122 nodes and 454 edges, whereas the intercropping treatment boasted 136 nodes and 585 edges (Figure 3A). This finding suggests that the bacterial co-occurrence network under intercropping was more intricate and interconnected. Conversely, the fungal network exhibited a slight decline, with the number of nodes and edges decreased from 131 to 90 and from 708 to 389, respectively, when comparing monoculture to intercropping (Figure 3A). Meanwhile, the topological analysis of the co-occurrence network revealed that the intercropping treatment led to lower Betweenness centrality, Closeness centrality, and Node degree compared with the corresponding monoculture network (Figure 3C). In conclusion, intercropping tea with *T. jasminoides* fostered bacterial aggregation in tea plantations, evidenced by a higher number of nodes and edges in the bacterial co-occurrence network (3.72:4.3). However, this aggregation was accompanied by a decrease in the centrality and connectivity of individual bacterial nodes.

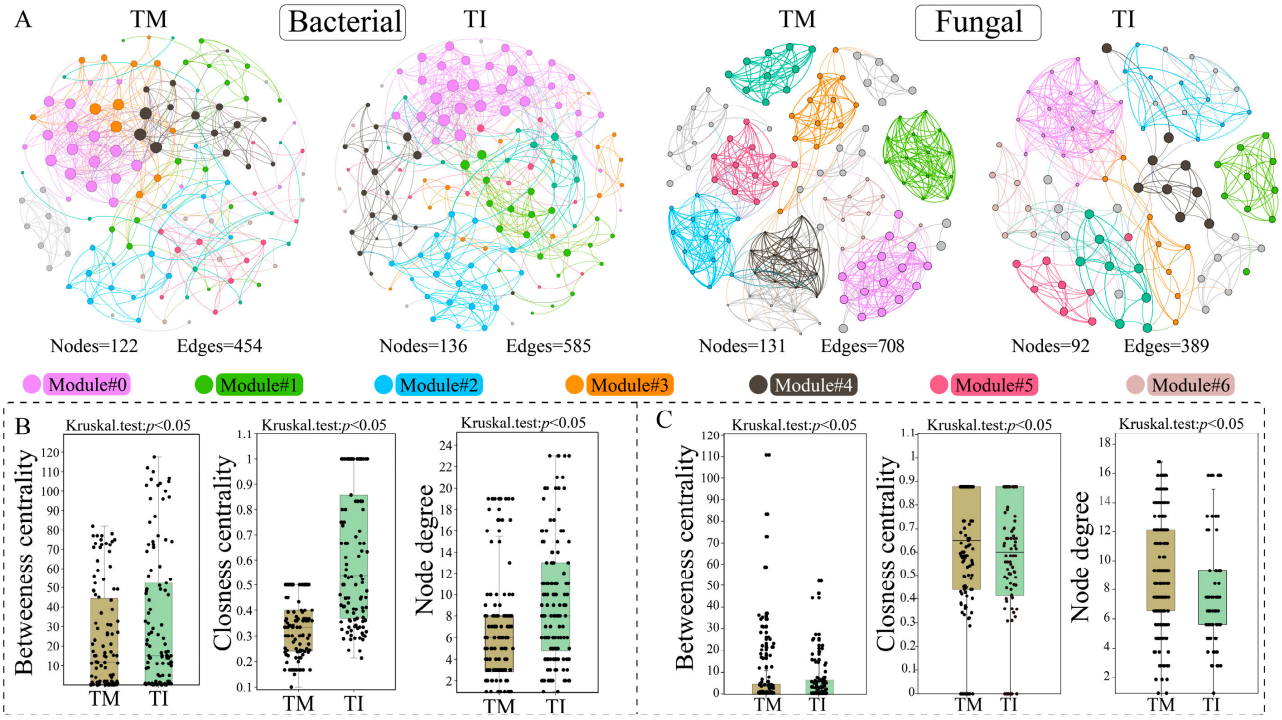

Figure 3. Soil microbial co-occurrence networks and their characteristics under different treatments. (**A**) Comparison of bacterial and fungal co-occurrence networks in soil under monoculture and intercropping systems in tea plantations. The networks were constructed based on the relative abundance correlation analysis among microbial genera. The color of each node corresponds to a specific microbial module. Connections between nodes indicate significant correlations, determined through a Spearman rank correlation test, with a significance level of $p < 0.05$ and a correlation coefficient greater than 0.70. Panels (**B**,**C**) depict the topological characteristics of the bacterial and fungal networks, respectively. These characteristics include Betweenness centrality, Closeness centrality, and Node degree.

3.4. Impact of Intercropping on Soil Microbial Community Structure

This study utilized Manhattan analysis to assess variations in the relative abundance of operational taxonomic units (OTUs) and to examine the influence of intercropping patterns on the soil microbial community in tea plantations (Figure 4A,D, Table S3). The results show substantial disparities in bacterial and fungal OTUs between intercropping areas and monoculture. Specifically, for bacteria, intercropping exhibited 410 significantly different OTUs compared with monoculture ($p < 0.05$). These OTUs were primarily distributed in the phyla Acidobacteria, Chloroflexi, and Proteobacteria. Notably, Acidobacteria exhibited a major downregulation of differentially abundant OTUs, accounting for 59.73% of the total significant differences. In Chloroflexi, the ratio of upregulated-to-downregulated OTUs was 42.72% to 36.98%, respectively. Conversely, Proteobacteria were dominated by upregulated OTUs, comprising 62.5% of the total significant differences. In the fungal realm, intercropping showed 250 significantly different OTUs compared with monoculture ($p < 0.05$). These OTUs were primarily distributed across the phyla Ascomycota and Basidiomycota. Within Ascomycota, the upregulated OTUs constituted 37.08% of the total OTUs, whereas the downregulated OTUs accounted for 43.26%. In Basidiomycota, the upregulated OTUs comprised 33.33% of the total OTUs, and the downregulated OTUs accounted for 50.88%.

The LEfSe (linear discriminant analysis effect size) analysis revealed a significant impact of intercropping teas and *T. jasminoides* on the soil microbial community (LDA > 3.5). Specifically, under the intercropping conditions, several key bacterial genera were enriched in the soil (Figure 4C,D). These included *Sinonmonas*, *Acidothermus*, and *Actinospica* from the phylum Actinobacteria; *Mesorhizobium*, *Nitrosospira*, *Burkholderia*, and *Sphingomonas* from the phylum Proteobacteria; *Granulicella* from the phylum Acidobacteria; and *Gemmatimonas* from the phylum Gemmatimonadetes. Notably, intercropping significantly boosted the abundance of these bacterial genera in the soil. Furthermore, in the fungal community, intercropping led to the enrichment in *Penicillium* and *Trichoderma* from the phylum Ascomycota. In contrast, compared with the monoculture (TM) treatment, the abundance of *Curvularia*, *Sagenomella*, *Purpureocillium*, *Aspergillus*, and *Fusarium* was significantly diminished.

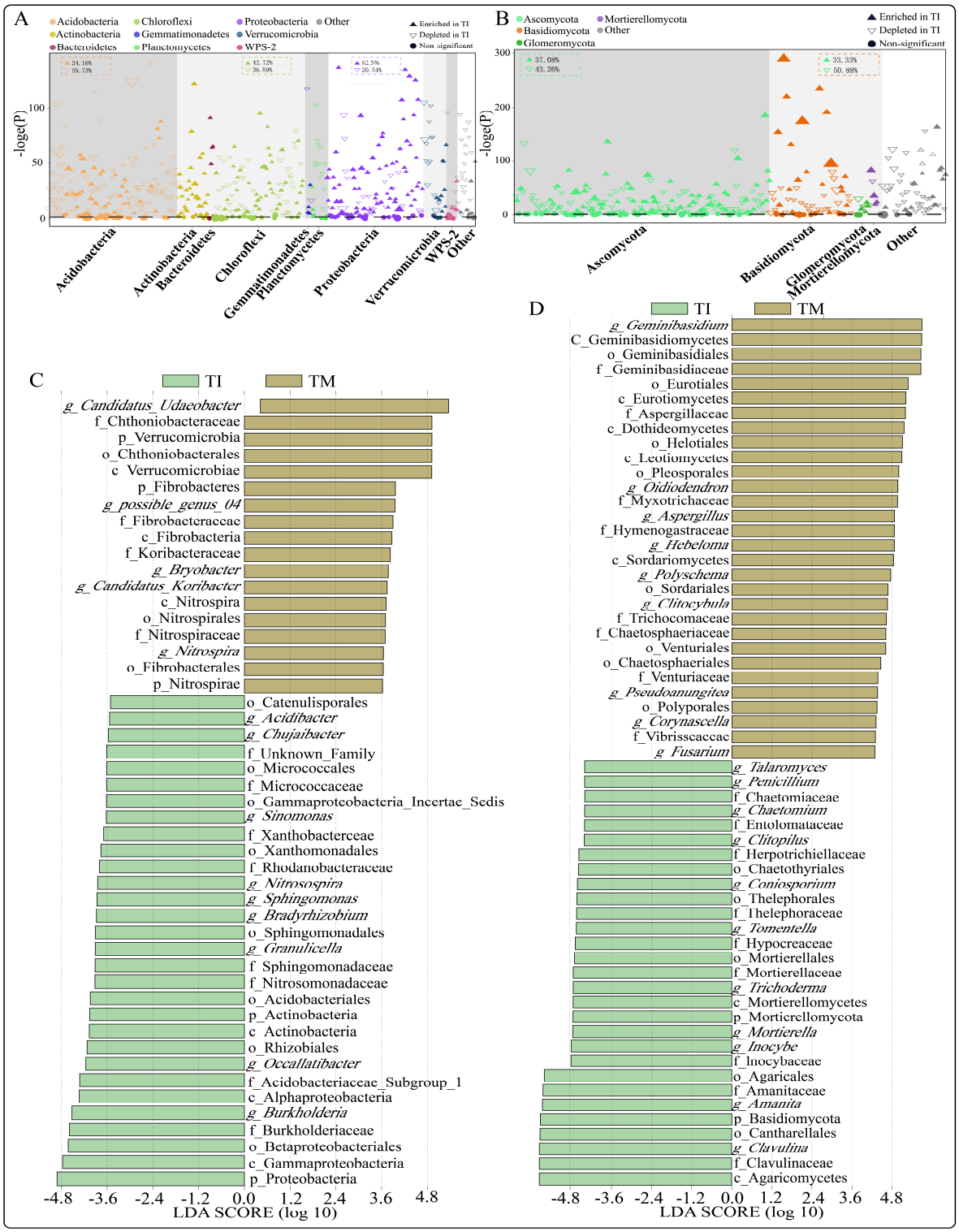

Figure 4. Classification characteristics of different bacterial taxa among the soil microbial communities under TI and TM treatments. The differential abundance of OTUs in the soil microbial communities under different treatments is displayed in Manhattan plots. Panels (**A**,**B**) depict enriched or depleted OTUs in the soil microbial communities of the samples. Each circle or triangle represents an OTU, with solid triangles indicating enrichment and empty triangles indicating depletion. OTUs with no significant change are represented by solid circles ($p > 0.05$). Panels (**C**,**D**) show the key differential bacterial (**C**) and fungal (**D**) genera in the microbial communities, with an LDA (linear discriminant analysis) score greater than 3.5.

3.5. Impact of Intercropping on Soil Bacterial Functions and Fungal Nutrient Modes

The FAPROTAX database, which relies on the current literature regarding cultured strains, serves as a tool for predicting ecological-related functions associated with prokaryotic branches such as genus or species [31,32]. By applying this database to functionally annotate soil bacteria across different treatments, we uncovered statistically significant variations in bacterial functions associated with material degradation ($p < 0.05$) (Table S4). Notably, these findings indicate predictive significance for all OTUs analyzed. In particular, the analysis revealed significant alterations in material degradation functions in bacteria exposed to intercropping treatments (Figure 5A). Specifically, there was a significant increase in the proportion of functions such as xylanolysis, methylotrophy, cellulolysis, hydrocarbon degradation, methanol oxidation, aliphatic non-methane hydrocarbon degradation, and aromatic hydrocarbon degradation. Furthermore, intercropping significantly enhanced functions related to nitrogen cycling, encompassing nitrogen fixation, nitrification, ureolysis, and nitrate reduction.

Based on data from the FUNGuild database, functional predictions were generated for the fungal community in the soil samples (Figure 5B, Table S5). These predictions encompassed the identification of various trophic modes among fungi, including symbiotrophs, saprotrophs, and pathogens. A notable trend emerged in the fungal community of intercropped soil, exhibiting a significant increase in the proportion of symbiotrophs and saprotrophs compared with their monoculture counterparts, while the proportion of pathogens was significantly lower ($p < 0.05$). Further analysis of the major fungal trophic modes revealed that intercropping had a substantial positive impact on the abundance of several fungal groups, such as lichenized fungi, epiphytic fungi, endophytic fungi, ectomycorrhizal fungi, plant saprotrophs, and soil saprotrophs. Conversely, the proportion of plant pathogens significantly decreased as a result of intercropping.

In summary, the intercropping practice of teas with *T. jasminoides* had a profound impact on the functional microorganisms present in the soil. Specifically, this practice significantly augmented the bacterial community's proficiency in executing crucial processes like material degradation and nitrogen cycling. Furthermore, intercropping led to a notable increase in the prevalence of co-occurrence functions among the fungal community, while simultaneously reducing the abundance of functions related to plant pathogens, compared with monoculture.

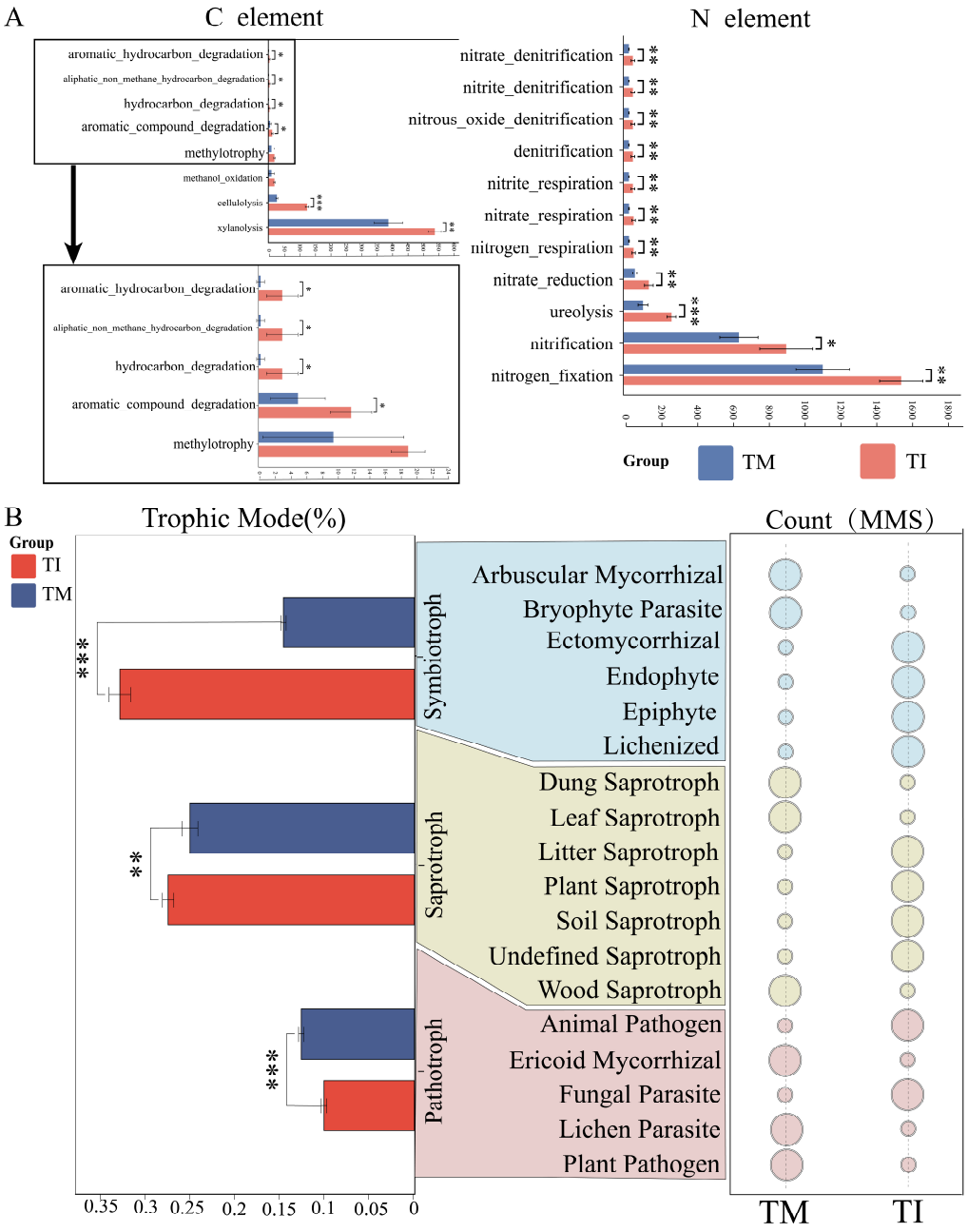

Figure 5. Functional prediction analyses of soil microbial communities under different treatments. (**A**) FAPROTAX analysis of bacterial communities; (**B**) FUNGuild analysis of fungal communities. In this context, the notation * denotes a significance level of $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$.

3.6. Impact of Intercropping on Soil Environmental Variables and Microbial Correlations

Using redundancy analysis (RDA), we demonstrated the influence of soil environmental variables on bacterial and fungal communities. Specifically, RDA1 and RDA2 collectively accounted for 96.7% of the variation in the bacterial community and 98.9% of the variation in the fungal community, as depicted in Figure 6A. Except for AN and AK, all other soil environmental variables exerted a significant impact on soil microbial communities. Notably, factors such as pH, CE, IE, ACPT, AP, and POD showed a significantly positive correlation with each other and exerted a more significant impact on the bacterial and fungal communities in the intercropping treatment. In contrast, the bacterial and fungal communities in monoculture soil were more influenced by UE and PPO.

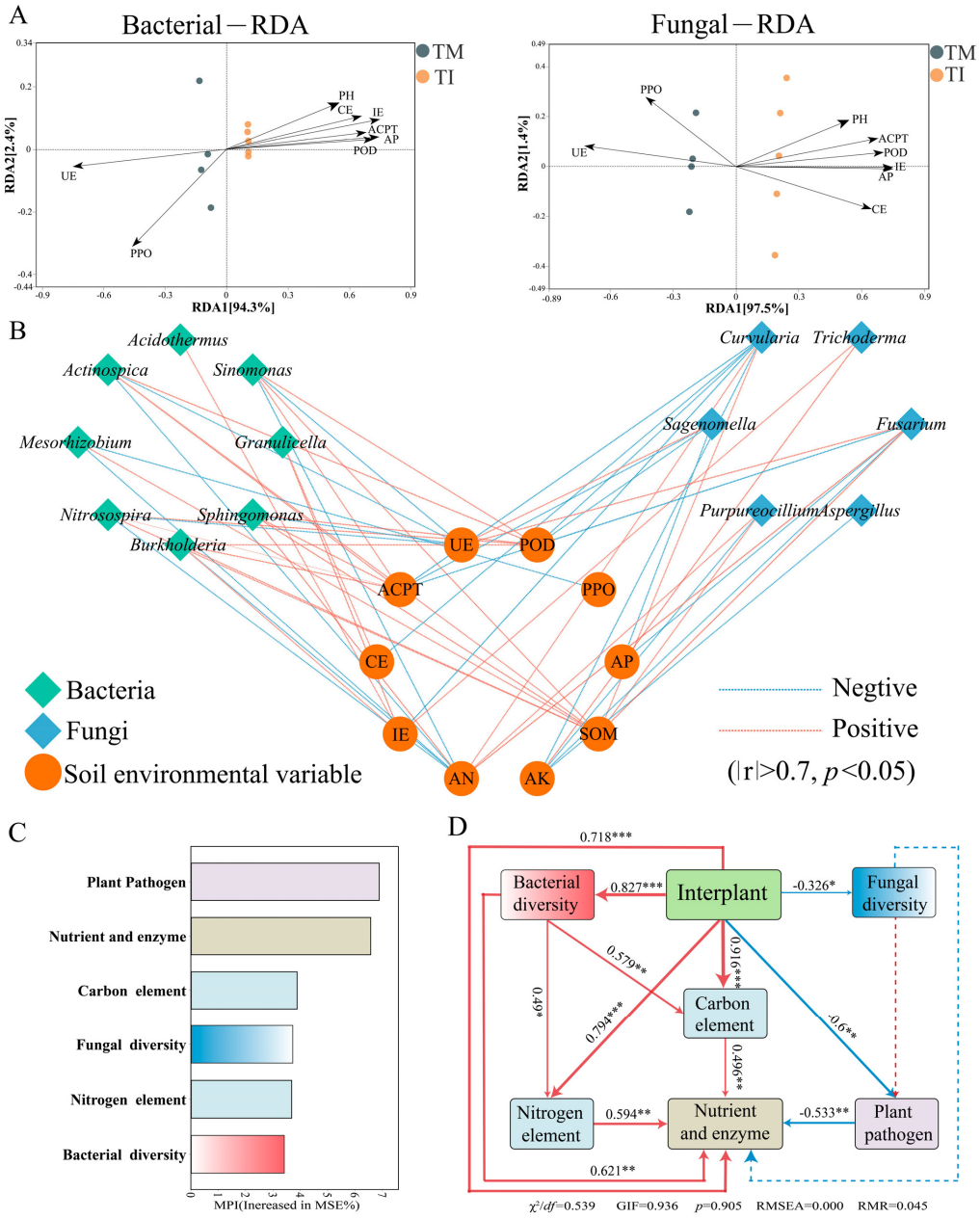

Figure 6. Analysis of the soil microbial community and interaction with the soil environment. (**A**) RDA, (**B**) correlation analysis between significantly different bacterial genera and soil factors based on Spearman algorithm ($|r| > 0.7$, $p < 0.05$), (**C**) random forest regression model analysis, and (**D**) Structural Equation Model analysis. In this context, the notation * denotes a significance level of $p < 0.05$, ** represents $p < 0.01$, and *** represents $p < 0.001$.

To further elucidate the relationships between key differential genera and soil environmental variables, we performed a Spearman correlation analysis (Figure 6B). Notably, only significant correlations with $|r| > 0.7$ and $p < 0.05$ were included for clarity. Among the bacterial genera, *Sinonmonas*, *Actinospica*, *Mesorhizobium*, and *Nitrosospira* exhibited a notable negative correlation with UE and PPO ($p < 0.05$), while displaying a significant positive correlation with other environmental factors. Similarly, most of the other key differential bacterial genera demonstrated a significant positive correlation with SOM, IE, AN, and other environmental factors. However, in the tea/*T. jasminoides* intercropping system, the majority of the differential fungal genera, except for *Trichoderma*, showed a

negative correlation with soil physicochemical factors. In contrast, *Trichoderma* displayed a significant positive correlation with IE and AP.

To assess the key environmental factors in the soil ecosystem under intercropping patterns, a random forest regression model was implemented, and the significance of each factor was determined by the magnitude of Mean Squared Error (MSE) values. As depicted in Figure 6C, the analysis revealed that the plant pathogens and the carbon element emerged as the most significant factors. Meanwhile, nutrients and enzymes, bacterial diversity, the nitrogen element, and fungal diversity exhibited relatively comparable weights.

By using the Structural Equation Model (SEM), we inferred the direct and indirect effects of microbial diversity and key functional microbial groups on environmental factors within the intercropping pattern. As displayed in Figure 6D, the intercropping pattern exerted a direct positive impact ($\lambda = 0.718$) on soil nutrients and enzyme activity. Additionally, it had direct positive effects on bacterial diversity ($\lambda = 0.827$), nitrogen cycle ($\lambda = 0.794$), and carbon cycle ($\lambda = 0.916$). Bacterial diversity, in turn, exhibited direct positive impacts on nitrogen cycle ($\lambda = 0.49$), carbon cycle ($\lambda = 0.579$), and soil nutrients ($\lambda = 0.621$). Furthermore, nitrogen cycle ($\lambda = 0.594$) and carbon cycle ($\lambda = 0.496$) showed varying degrees of direct positive effects on soil nutrients. In contrast, the intercropping pattern had a direct negative impact ($\lambda = -0.6$) on plant pathogens, which subsequently exerted a direct negative impact on soil nutrients ($\lambda = -0.533$).

4. Discussion

4.1. Intercropping Significantly Influences the Microbial Community Structure in Tea Soil

The plant microbiome, often labeled as the second genome of plants, plays a crucial role in regulating growth, development, and overall vitality in host plants [33,34]. In recent years, there has been growing recognition that the advantages of intercropping are not solely determined by ecological niche diversity and nutrient absorption complementarity; rather, they are profoundly influenced by soil microbes and plant secretions. Particularly, the indirect yet pivotal role of soil biological interactions and plant–soil feedback mechanisms cannot be overstated [35,36]. As intercropping remains an essential agricultural practice for current and future agricultural progress, it is imperative to conduct a thorough investigation into the dynamic changes in soil microbial community structure and functionality under intercropping cultivation.

In this study, two cropping patterns were implemented: tea monoculture and tea intercropped with *T. jasminoides*. By utilizing high-throughput sequencing technology, we analyzed the soil microbial community structure under these different cropping patterns. The results revealed that intercropping with *T. jasminoides* fostered a distinct microbial community structure in tea soil (Figure 2). Soil serves as a habitat for microorganisms, with intricate relationships flourishing [37]. Previous studies have proposed that increasing the diversity of soil bacterial communities enhances the association of soil microbial species, thus improving soil ecological stability [38–40]. Consistent with these findings, we observed a significant increase in bacterial community diversity and richness under intercropping (Figure 2A). Furthermore, the topological analysis of the co-occurrence network revealed that Betweenness centrality, Closeness centrality, and Node degree were higher in the intercropping network compared with the monoculture co-occurrence network (Figure 3B). These results indicate that intercropping tea with *T. jasminoides* enhances the stability of correlations between bacterial species and enriches key microbial groups. However, the fungal diversity index showed a slight decrease. Similar trends have been observed in other studies, where intercropping with various legume green manures did not lead to significant changes in the Shannon index of root fungal and archaeal communities [41]. Additionally, a study on wheat/watermelon intercropping showed that microbial community diversity did not increase [42]. Notably, research has suggested that soil ecosystem functions are more dependent on functional diversity rather than taxonomic diversity [43]. Therefore, it is plausible to speculate that there is no direct relationship between the establishment of intercropping dominance and changes in fungal diversity.

4.2. Intercropping Cultivation Improves Soil Nutrient Levels by Promoting Soil C and N Cycling

In this study, a significant increase in SOM was observed after the implementation of the intercropping treatment, compared with the tea monoculture treatment (Figure 1). Additionally, several soil nutrient indicators, including AK, AP, ACPT, EC, and POD, all exhibited significantly enhancement under the intercropping treatment. These findings indicated that intercropping tea with *T. jasminoides* improved soil nutrient levels, which is consistent with previous research results [44]. Therefore, the tea/*T. jasminoides* intercropping system serves as an effective means to promote soil fertility nutrient availability.

Soil microorganisms play a crucial role in the transformation and cycling of soil organic matter, as they facilitate the breakdown of insoluble organic compounds into soluble ones [45]. Cellulase, mainly derived from soil microorganisms, is involved in releasing and supplying available nutrients within the soil ecosystem [46]. Preceding investigations have highlighted that intercropping systems, such as soybean and corn, can significantly increase soil organic matter and pH [36]. In line with these findings, our study observed a marked increase in pH, soil enzyme activity, and organic matter content, suggesting a potential link with microbial–organic matter cycling under *T. jasminoides* intercropping. The conditions fostered by intercropping, including root secretions and the accumulation of litter, have a significant impact on specific functional microbial groups within the soil. Our study revealed that there were significant impacts on the taxonomic structures of bacteria (Proteobacteria, Chloroflexi, and Acidobacteria) and fungi (Ascomycota and Basidiomycota) (Figure 4). It is well established that soil carbon cycling is primarily driven by soil microorganisms [47]. Proteobacteria, for instance, is crucial to degrading organic compounds, including xenobiotic, recalcitrant aromatic compounds, and complex pollutants [10,48,49]. Ascomycetes and basidiomycetes, as primary decomposers among soil fungi [50,51], contribute significantly to the degradation of organic matter, such as lignocellulose, with basidiomycetes playing an important role [52,53].

Further analysis showed a significant increase in the abundance of nine key bacterial taxa under intercropping treatment. These taxa included *Sinonmonas*, *Acidothermus*, *Actinospica*, *Mesorhizobium*, *Nitrosospira*, *Burkholderia*, *Sphingomonas*, *Granulicella*, and *Gemmatimonas* (Figure 4C,D). Among them, *Acidothermus* and *Sinonmonas*, both belonging to the phylum Acidobacteria, are beneficial genera capable of efficiently degrading glucose and cellulose, thus promoting plant growth through organic nutrient utilization. *Actinospica*, commonly present in soils, has the potential to produce natural antibiotics [54]. *Sphingomonas*, a strictly aerobic bacterial genus, exhibits remarkable degradation capabilities for aromatic compounds [55]. The enrichment in these bacterial taxa under intercropping conditions indicated enhanced degradation of multi-level organic substances in the soil carbon cycle, leading to soil enrichment with fixed carbon sources and providing more abundant nutritional supply for tea growth. Notably, *Burkholderia* is recognized as a plant growth bacterium that typically harbors the *nifH* gene, crucial to plant nitrogen fixation [56,57]. *Mesorhizobium*, well known for its nitrogen-fixing abilities, is commonly found in root nodules, forming a highly synergistic relationship with plant roots [58]. Additionally, *Gemmatimonas* has been shown in numerous studies to possess significant capabilities in nitrogen transformation and fixation [59,60]. With regards to nitrogen cycling-related microorganisms, this study also observed an increase in the abundance of *Nitrosospira* under intercropping conditions. *Nitrosospira* itself is an important genus involved in soil nitrification [61]. The alterations in the abundance of these key bacterial taxa suggest that intercropping tea with *T. jasminoides* can enhance soil nitrogen fixation functions by enriching specific, vital microbial groups.

The functional predictions of the soil microbial community further confirmed the positive effect of intercropping on soil carbon (C) and nitrogen (N) cycling. The study revealed a marked enhancement in various soil carbon cycle functions related to organic matter degradation, such as xylanolysis, methylotrophy, and cellulolysis. Additionally, N cycling functions such as nitrification, denitrification, and nitrogen fixation were significantly augmented under intercropping compared with monoculture (Figure 5B). The functional

predictions for the fungal community also demonstrated the beneficial effect of intercropping on the C cycle. The proportions of saprotrophic fungi, including litter saprotrophs, plant saprotrophs, soil saprotrophs, and ectomycorrhizal fungi, significantly increased in the fungal community. This increase in saprotrophic fungi indicated an enhancement in the soil fungal community's ability to degrade organic matter and self-toxic compounds [62]. Among the enriched fungal taxa under intercropping, *Penicillium* and *Trichoderma* stand out, having been extensively studied for their roles in decomposing and utilizing organic matter, thereby promoting ecological balance [63,64]. This finding may be closely related to the observed improvement in microbial functions in the carbon cycle. Both correlation analysis (Figure 6B) and Structural Equation Model (SEM) analysis (Figure 6D) further confirmed the close relationship between the abundance of these fungal genera and the growth of most soil physicochemical factors. Overall, the intercropping pattern exerts a significant influence on bacterial diversity, fostering the N and C cycles in the soil, ultimately exerting a positive impact on soil nutrients and enzyme activity [65,66]. The increased root exudates and litter input into the soil, resulting from tea/*T. jasminoides* intercropping, provided ample substrate for microbial activity, significantly affecting soil substance and nutrient cycling. In conclusion, by enriching specific functional microbial groups, tea/*T. jasminoides* intercropping accelerated the release and recycling of soil nutrients.

4.3. Intercropping Cultivation Suppresses Soil Pathogenic Fungi

Previous studies have reported the rhizosphere effect of crop intercropping of suppressing soil pathogenic fungi. For instance, *Pseudomonas fluorescens* ZL22 possesses a well-established degradation pathway, efficiently breaking down high concentrations of PHBA and PA, thereby mitigating the self-toxicity effect on tea plants [7]. Similarly, in soybean/corn intercropping, corn root exudates effectively hindered the growth of *Cylindrocladium parasiticum*, the causative agent of soybean red crown rot, thereby enhancing soybean resistance to the disease [66]. The current study reveals a significant change in the abundance of pathogenic fungi in the fungal community, with a notable decrease in the abundance of *Curvularia*, *Purpureocillium*, *Aspergillus*, and *Fusarium* (Figure 4D) under tea/*T. jasminoides* intercropping. Numerous studies have demonstrated that *Curvularia* can infect various plants, resulting in diseases such as root rot, flower decay, and leaf blight [67,68]. Aspergillus is widely distributed in nature and can cause various forms of mold decay, posing a serious threat to plants [68]. *Fusarium*, as a globally distributed pathogenic fungus, commonly exists in the soil and can infect various plants, significantly affecting crop yield and quality [68,69]. Furthermore, several genera with biocontrol functions, such as *Purpureocillium*, *Penicillium*, *Talaromyces*, and *Trichoderma*, showed a marked increase in abundance under intercropping conditions [70,71]. A more thorough examination of the predicted functions of the fungal community revealed a significant reduction in the proportion of plant pathogens under intercropping conditions compared with monoculture (Figure 5D). Moreover, both the random forest regression model and the Structural Equation Model highlighted plant pathogens as the most crucial factor under different treatments (Figure 6C). Additionally, the intercropping model exhibited a significant negative impact on plant pathogens (Figure 6D). Consequently, tea and *T. jasminoides* intercropping can induce significant changes in the composition of plant pathogens within the soil microbial community, potentially suppressing pathogenic microbes and enhancing plant disease resistance. The inhibitory effect of tea/*T. jasminoides* on pests and diseases could be achieved by altering root exudates to modulate the soil microbial community. Tea/*T. jasminoides* intercropping not only influences the composition of root exudates but also enhances beneficial microorganisms and suppresses pathogenic fungi. It is worth noting, however, that the experimental tea garden in this study is representative of the southern mountain tea gardens, and variations in land types can significantly impact plant growth. In addition, the intricate interplay among plants, root secretions, and microbes necessitates a deeper investigation. Consequently, future research should further explore the mediating

role of different land types and root exudates in plant–soil microecology, so as to more comprehensively reveal the interaction mechanisms in tea/*T. jasminoides* intercropping.

5. Conclusions

The intercropping of tea with *T. jasminoides* significantly enhanced the nutrient cycles of nitrogen (N) and carbon (C) in the soil, leading to improved soil nutrient conditions. This intercropping practice induced significant changes in the functional microbial structure within the tea plantation soil, particularly among the functional genera responsible for substance degradation and soil nitrogen cycling. Notably, bacteria such as *Burkholderia*, *Mesorhizobium*, and *Gemamatimonas*, which are associated with nitrogen cycling, underwent significant enrichment. In contrast, pathogenic fungi, like *Aspergillus*, *Fusarium*, and *Curvularia,* displayed a notable decrease in abundance. Functional predictions indicate that tea/T. *jasminoides* intercropping increased the proportion of nitrogen and carbon cycling functions mediated by N and C nutrients in the soil. Moreover, the reduction in plant pathogens, representing plant pathogenic fungi, significantly enhances ecological stability in tea/*T. jasminoides* intercropping systems. Consequently, the intercropping of *T. jasminoides* with teas in tea plantations serves as a sustainable cultivation model and an effective strategy for preserving soil health and promoting sustainable production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14061261/s1, Figure S1: Experimental area, Table S1: Information on primers used in this study, Table S2: Analysis of co-occurrence networks of different soil microbial communities, Table S3: Manhattan analysis of microbial community structure differences, Table S4: Bacterial community function predicted by FAPROTAX, Table S5: Prediction of fungal community function based on FUNGuild database, Table S6: Prediction of fungal community function based on FUNGuild database.

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