Effects of long-term intercropping of pecan (Carya illinoinensis) on bacterial community in tea rhizosphere soil and tea quality

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Abstract

Using the 16S rDNA high-throughput sequencing, the effects of a nested pecan in a typical tea garden on soil bacterial group species composition and marker species were comparatively analyzed to determine whether the nested pecan contributed to an increased soil microbial diversity in the tea plant rhizosphere. We explored the effects of the underlying mechanisms of this complex ecosystem on tea quality by determining soil physicochemical properties and tea quality under two types of planting modes, intercropping of pecan versus pure forest. Our observations indicated that Allorhizobium, Neorhizobium, Pararhizobium, Rhizobium, and Enterobacter, with pollution-degrading effects, were enriched in the soil bacterial communities of interplanting pecan in the tea garden model. There was a significant enrichment of prebiotic functional bacteria, such as Pedosphaeraceae and the Coriobateriaceae_UCG-002 genus, which has growth stimulation and disease resistance, while Chloroflexi and Firmicutes, which dominate denitrification in the soil, were inhibited. The soil microbial co-occurrence network increased in complexity and decreased in mutual exclusivity, and the complex interactions among bacterial populations tended to be stable. The phenol ammonia ratio in tea leaf pieces was lower and the comprehensive quality evaluation of tea was improved in the intercropping model. We conclude that the soil microbial community composition under this complex model may not only promote material cycling in microecosystems but also effectively reduce the transmission risk of soil-borne diseases. The tea rhizosphere soil microbial community structure could be rebalanced and shifted toward a more favorable tea quality formation through the introduction of pecan into the tea plantations.

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Keywords: Forest tea intercropping; Soil microbial community; Physical and chemical properties of soil; Composition of plantation; Microecological balance

1 | INTRODUCTION

Tea is the leaf of *Camellia sinensis* , a plant of the*Camellia* genus in the *Camelaceae* family, and tea trees mainly grow in the southern subtropical hills of China, where the local soil is dominated by acidic red soil with low nitrogen content and little organic matter. Massive application of chemical fertilizers may cause degradation of the soil ecosystem, although it can increase soil nutrient content and improve tea yield in the short term (Xv et al., 2008). However, the unique ecological environment formed by the long-term cultivation of tea plants has an inhibitory effect on local soil microbial diversity (Xue et al., 2007). Therefore, how to achieve safe and efficient production and sustainable development of tea through ecological management measures is a concern for the industry at present. Because of the wet and shade tolerant characteristics of tea trees, the artificial introduction of a variety of plants, with ecological niches that are different from those of tea trees, through interplanting of other crops can give full play to the complementary characteristics of plants, improve the ecological environment structure of tea gardens, and improve tea yield and quality. *Carya illinoinensis*(wangenh.) K. Koch, also known as the American pecan, is a deciduous tree of the Pecan genus in the *Jugaceae* family. It is one of the world's famous dried fruits and an excellent tree species for its dual use. It is naturally distributed in the southern United States and northern Mexico, and its commercial cultivation is mainly in the Mississippi Valley alluvial plain (Li et al., 2011), whose central production region coincides perfectly with the tea tree adaptive climate zone. The pecan and tea composite ecosystem follows the principles of optimal niche configuration, using woodland spaces to create stratified forests and substantially improve tea garden space use and productivity. Therefore, interplanting pecan in tea gardens is a new agroforestry management mode with considerable development potential. There have been many previous discussions on the modes of operation of deciduous woody trees from lariats in tea plantations (Zhou et al., 1995; Wang et al., 2014), but most of them focused on the changes in the chemical composition of tea. The lack of studies on the differences in soil microbial diversity in the tea rhizosphere and the mechanisms of tea quality formation in response to changes in the soil flora creates uncertainty about the evaluation of the ecological effects and economic benefits of the composite ecosystem of tea plantations.

Soil microbial diversity is closely related to the ability of a plantation ecosystem to resist external disturbances, and a healthy and stable soil microbial community plays an important role in preserving soil fertility, improving soil structure, buffering soil pollution, and synergizing plant growth and other ecological service functions (Yang et al., 2000). Heterotrophic microbial communities colonizing soils are involved in regulating

several key ecological processes that control the carbon-nitrogen cycle of the ecosystem, and they potentially embody a mechanistic link between plant diversity and ecosystem function. Few studies have revealed the mechanisms of influence between plant diversity and soil microbial diversity, but many scholars have been committed to exploring this aspect through research. Spehn (Spehn et al., 2000) showed that the number of soil microorganisms was linearly related to the number of plant functional groups, and the number of soil microorganisms was significantly reduced by 15% when legumes were missing from the functional groups. The ratio of soil microbial carbon to soil organic carbon also decreases with the loss of plant species and a reduction in the number of plant functional groups. Porazinska (Porazinska et al., 1998) found that some bacteria and nematodes in the root soil of C3 and C4 plants of different combinations react strongly to endemic plant species by investigating the aboveground and underground diversity of plant communities in the natural grassland of Kansas. The interaction between aboveground and underground organisms provides important feedback for the regulation process of the ecosystem: the composition of biological communities is determined by the availability of resources that restrict plant growth. As the material and energy source of the degrader, the resources available to the soil microbial community are limited by the chemical composition of the litter (Hu et al., 2005). Owing to the difference in the chemical composition of plants, changes in plant diversity lead to organic changes in the secondary metabolite products and litter, thus affecting the composition and function of heterotrophic microbial communities (Jiang et al., 2011). Previous studies have focused on the relationship between tea plant growth and soil microbial diversity in pure tea gardens (Xv et al., 2007; Xue et al., 2007; Wang et al., 2012; Lin et al., 2013), and did not fully consider the effect of changes in aboveground plant diversity caused by interplanting in tea gardens on soil microbial diversity and ecosystem functions. In this study, tea produced in Songyang, Zhejiang Province was taken as the experimental object. Pecan interplanting (T) was conducted in typical tea gardens for 8 years, and the pure tea garden (CK) was used as a blank control. By comparing the relative abundance, diversity, and composition differences of soil microorganisms under the two types of planting modes, the effects of soil microbial community changes on soil physical and chemical properties and tea quality were preliminarily evaluated. This study can clarify whether the long-term interplanting of pecan in tea gardens has an effect on the rhizosphere soil flora of tea plants, and further clarify the core bacteria related to tea growth and quality formation and their mechanisms. This study will not only help to formulate safe and efficient production strategies for the construction of pecan and tea complex ecosystems, but will also provide more direct theoretical support for the development of the tea garden stereoscopic planting industry.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling

The test materials were taken from the test base at Dongguan Village, Zhaitan Township, Songyang County, Zhejiang Province, with geographic coordinates of E28.472-28.474; N119.413-119.417. The test site has a subtropical monsoon climate with four distinct seasons and abundant rainfall. The annual average temperature is 17.7 °C, the annual precipitation is 1700 mm, the frost-free period is 236 days, and the annual average sunshine hours are 1,840 h. The soil of the test site is acidic red soil, fertile, and has good site conditions.

At each site, we randomly selected five sampling points in the plot of each planting mode. Within the vertical ground projection of the crown (1.5 m × 1.5 m) of the pecan, five points were randomly selected for sampling in June 2022. One hundred and fifty grams of tea leaf mixed samples were collected along the vertical projection around the crown, and each treatment was repeated five times. A total of 10 tea samples were collected. Before sampling, the top litter layer was removed and the soil collector was wiped. Approximately 0–20 cm of soil layer was collected from 10 cm around the root of the tea tree at each point, and the soil collected from each treatment group plantation was randomly divided into five samples. A total of 10 soil samples were collected from two locations using the same method. Fresh soil was sorted with the hand to remove roots and stones. All samples were further divided into two groups and placed in sterile bags. The first part of the soil was immediately frozen in liquid nitrogen, stored on dry ice, transported back to the laboratory, and then refrigerated at -80 degC until DNA was subsequently extracted. The other group was passed through a 2-mm sieve and stored at 4 degC to determine the physicochemical properties of the

soil.

2.2 | Soil physicochemical properties

The physical and chemical properties of soil include pH, organic carbon, total nitrogen, nitrate nitrogen, ammonium nitrogen, total phosphorus, inorganic phosphorus, organic phosphorus, available phosphorus, total potassium, available potassium, available potassium, slow-acting potassium, iron, magnesium, calcium, manganese, boron, zinc, copper, and cesium. The SPSS 19.0 software was used for the single factor variance (ANOVA) test to screen out the physical and chemical indicators of the soil with significant differences between treatments for subsequent analysis.

2.3 | DNA extraction and high-throughput sequencing

Bacterial DNA was extracted using a Qiagen DNeasy blood and tissue kit, followed by the determination of DNA concentrations and size distributions. The extracted DNA from each sample was used as input for library preparation using a TruSeq Nano DNA LT Library Prep kit. Prior to sequencing, an Agilent BioAnalyzer was used to evaluate library quality using an Agilent High Sensitivity DNA kit. After the libraries had been validated, they were quantified using a Quant-iT PicoGreen dsDNA assay kit (Promega). PCR was used to amplify the V5–V7 hypervariable regions of the bacterial 16S rRNA genes. 16S rRNA gene amplification was performed using the forward primer 799F (5′-AACMGGAT-TAGATACCCKG-3′) and the reverse primer 1193R (5′-ACGTCATCCC-CACTTCC-3′). Paired-end sequencing (2 × 300 bp) of the quality-validated samples was conducted on the Illumina MiSeq platform using the MiSeq reagent kit V3 (600 cycles) (Jiang et al., 2021). A target fragment size of 200–450 bp was used for library construction, which was performed at the Personal Biotechnology Company (Shanghai, China).

A 16S rRNA sequence quality filtering was conducted using Cutadapt (v.1.9.1;*https://cutadapt.readthedocs.io/en/stable/)*. Quality-filtered paired-end reads were then merged using UCHIME (*http://www.drive5.com/usearch/manual/uchime_algo.html*) (Edgar et al., 2011). The high-quality sequences were clustered into operational taxonomic units (OTUs) at the 97% nucleotide similarity level using UPARSE (Wang et al., 2009). Chimeras were identified and removed from the dataset. DADA2 (Bolyen et al., 2019) was used to BLAST representatives of OTUs against the Silva database (Wang et al., 2007) (*https://www.arb-silva.de/*). OTU subsampling was conducted to facilitate comparison among the samples. Spearman correlation analysis was conducted for OTU abundances across the samples, and only robust (Spearman's r of > 0.6 or r of < 20.6) and significant ($P < 0.01$) correlations were retained for network analysis. Network analysis of OTU abundances was conducted using the Psych software for R (Faust et al., 2012), followed by visualization with Gephi (Bastian et al., 2009). Alpha diversity indices (Shannon diversity, Simpson diversity, ACE richness, Chao1 richness, and whole tree PD) were calculated using QIIME2 (Bastian et al., 2009). Beta diversity values (Bray-Curtis distances) were analyzed using nonmetric multidimensional scaling (NMDS) (Bolyen et al., 2019). The significance of the bacterial community variation among the three regions was evaluated with an analysis of similarities (ANOSIM) test. To evaluate the most discriminatory taxa across samples, the relative abundances of bacterial taxa at the genus level were assessed using the random forest package (v. 4.6-14) for R with default parameters (Wang et al., 2007). The physical and chemical properties of the soil and soil microbial community were calculated using the R software (v. 4.1.3) (Liaw et al., 2002; Sunagawa et al., 2015). Alpha diversity, signature bacteria genus, and correlation analysis were carried out. Spearman correlation coefficient was used to compare the environmental factors in pairs, and then the linkET, ggplot2, dplyr, and ggtext packages were used to plot the Mantel test and correlation heat map.

2.4 | Detection of tea quality index

Tea quality indicators include chlorophyll (Chl, mg/g), tea polyphenols (GTP,%), and catechin components (%), which include catechin (C), epicatechin (EC), gallic acid (GA), gallic catechin (GC), epigallocatechin (EGC), catechin gallate (CG), epicatechin gallate (ECG), epigallocatechin gallate (GCG), and epigallocatechin gallate (EGCG). The alkaloids (%) include theobromine (TB), theophylline (THEO), and caffeine (Caf). The free amino acids include aspartic acid (Asp)threonine (Thr), serine (Ser), glutamic acid (Glu),

theanine (Thea), glycine (Gly), alanine (Ala), valine (Val), cystine (Cys), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr) phenylalanine (Phe), gamma aminobutyric acid (GABA), lysine (Lys), histidine (His), arginine (Arg), and proline (Pro).

The OPLS-DA method was used to analyze all tea quality indicators with Simca14.1 and the ropls package of R software. After establishing a reliable model, the indicators with VIP values greater than 1 were selected. Then, SPSS 19.0 was used to establish a matrix around the above indicators and samples, and used to conduct factor analysis. The R software package (v. 4.1.3) was used to analyze the indicators and alpha diversity of the soil microbial communities and the signature of the bacterial genera. A correlation analysis was carried out, and the Mantel test and correlation heat map were drawn using the method described in 2.3.

3 | RESULT

3.1 | Intercropping of pecan and tea tree changes the physical and chemical properties of tea rhizosphere soil

The physical and chemical properties of tea soil under different interplanting conditions are different. Specifically, soil nitrate nitrogen (NO_3), boron (B), and magnesium (Mg) increased significantly under the condition of interplanting of pecan and tea, while total phosphorus (TP) and inorganic phosphorus (AP) decreased significantly ($P < 0.01$). Pearson correlation analysis found (Figure 2a) that NO_3 was significantly negatively correlated with TP and significantly positively correlated with B and Mg ($0.01 < P < 0.05$). B was significantly negatively correlated with TP and AP ($P < 0.01$). TP was negatively correlated with Mg and positively correlated with AP.

3.2 | Interplanting of pecan and tea optimized the relationship among the soil microecosystem members.

The co-occurrence network diagram was used to explore the relationship between microorganisms. The genera with relative abundance greater than 0.05% were selected for Spearman correlation analysis, and a correlation coefficient greater than 0.6 and a P-value of less than 0.01 were screened to build a correlation network. The topological characteristic parameters were calculated and compared with the Erdös–Réyni random network graph of the same size. The parameters were higher than those of the random network: the average path distance c was 0.380/0.319, the average clustering coefficient was 0.071/0.060, and the modularity index was 2.943/2.635. This shows that the microbial network in this experiment is composed of closely connected nodes and forms a "small world" topology (Table 1). The nodes in the network diagram are mainly divided into 10 phyla; Proteobacteria, Actinobacteria, Acidobacter, Chloroflexi, Bacteroides, and Firmicutes are the main six phyla, accounting for approximately 90% of all nodes. These nodes form four main modules, and nodes in different modules may play different functions; however, there is a strong ecological connection (Figure 1a).

The co-occurrence network analysis showed that the species composition of the soil microbial community changed significantly under different interplanting conditions. In the tea monoculture soil, *Proteus*(43.59%), *Actinomyces* (18.80%), and *Acidobacter*(12.82%) dominated, while under the condition of walnut tea interplanting, the abundances of *Proteus* (43.98%) and*Actinomyces* (22.29%) increased, while the abundance of*Acidobacter* (9.04%) decreased. With an average degree of 13.40–18.17, the number of hub nodes (nodes with high degree values of > 60 and closeness centrality of > 0.3) in the network increased (Figure 1a; Table 1). Therefore, the diversity of tea garden plants affects the soil bacteria alpha diversity (i.e., Chao 1 and the observed richness indicators), and network complexity (the higher the average degree, the higher the corresponding network complexity) has a significant effect (Figs. 1a and 1b).

For the physical and chemical properties of the soil, soil NO_3 is significantly positively correlated with the Chao 1 index and observed richness, the relative content of B is extremely significantly positively correlated with Chao 1 index, and the relative content of Mg is significantly positively correlated with observed richness (Figure 2a). A NMDS analysis of the soil microbiome was conducted based on the Bray-Curtis distance, and

the results show that soil microbiome samples with different planting patterns overlap in species composition, but could be roughly divided into two groups. Genus differences of the gut microbiota of the two groups were compared by wilcox.test (Figure 3), and the random forest classification method was used to determine the soil marker microorganisms in the planting garden under different interplanting modes. It was found that the interplanting of pecan and tea tree did not only change the proportion of the dominant endophytic bacteria in the rhizosphere soil of tea plants, but also enriched the species of *Dyella* , *Allorhizobium* ,*Neorhizobium* , *Pararhizobium* , *Rhizobium* ,*Pedosphaeraceae* (genera_unclassified),*Coriobacteriaceae_UCG-002* , and *Enterobacter* , and other beneficial bacteria with growth-promoting functions, such as phosphorus hydrolysis, nitrogen fixation, and disease inhibition (Figure 1d). A correlation analysis of the relative abundance of these five genera and the soil physical and chemical indicators show that the accumulation of NO_3 is significantly positively correlated with the increase in the relative abundances of four genera except for the Dyella.

3.3 | Interplanting of pecan and tea tree significantly improves tea quality.

A supervised pattern recognition method, orthogonal partial least squares discriminant analysis (OPLS-DA), was introduced. Twenty-two personality indicators were selected as the X variables in addition to tea quality under two interplanting methods as the variables for the OPLS-DA analysis. Ten tea samples have no outliers and could be distinguished (Figure 4a). Where R2 (X) = 0.527, the closer R2 (X) is to 1, the more stable the model is; where R2 (Y) = 0.923, the larger R2 (Y) is, the stronger the explanatory power of the model is, which could explain 92.3% of the original data. Q2 = 0.726 and Q2 > 0.7, indicating that the model has strong prediction ability. The variables of the categorical Y-matrix defined at the time of model building were randomly permuted 200 times, with R2 = 0.710, Q2 = -0.464, and Q2 < 0, indicating that this OPLS-DA model was reliable and had no overfitting phenomenon, and this model could be used for discriminant analysis of the respective categories. Figure 4b the variable importance for the projection (VIP) score plot obtained for the OPLS-DA analysis, in which it is generally accepted that variables with VIP of > 1.0 have an important role in the discrimination process. A total of 13 indicators with VIP greater than 1.0 were obtained; in order of VIP value magnitude, they are alanine (Ala), tea polyphenol (Gtp), glycine (Gly), threonine (Thr), leucine (Leu), tyrosine (Tyr), lysine (Lys), theanine (Thea), theobromine (Tb), γ-aminobutyric acid (GABA), isoleucine (Ile), serine (Ser), and aspartic acid (Asp).

The matrix of 13 evaluation indexes with 10 tea samples constituting 13 × 10 was used for factor analysis, and the comprehensive scores of each sample were obtained and presented in the form of box plots (Figure 4c). As shown in the figure, the comprehensive score of tea quality under the interplanting of pecan and tea tree is significantly higher than that under the pure tea forest ($P < 0.01$).

Among the tea quality indexes, soil Chao 1 index showed a significant positive correlation with Ser, Leu, and Tyr; observed richness with Thr, Ser, Thea, Ala, Ile, Leu, Tyr, and Lys; and Shannon index with Thr, Thea, Ala, Leu, Tyr, and Lys (Figure 5a). The results of the correlation analysis between the relative abundances of five soil marker microorganisms and tea quality indexes showed there is a significant positive correlation (in all cases, $P < 0.01$) between the genus *Dysseria* and Ser and Tyr; the genera *Allorhizobium* ,*Neorhizobium* ,*Pararhizobium* , *Rhizobium* and Ala and GABA;*Pedosphaeracea* and Ser, Thea, and Tyr; the genus*Coriobacteriaceae_UCG-002* and TB, Asp, and Ala; and the genus*Enterobacter* and TB, Ala, and GABA (Figure 5b).

4 | DISCUSSION

The microbial community was able to respond rapidly to the environmental changes induced by planting patterns, with pecan as a high deciduous tree, producing a large amount of litter to cover the surface while providing understory shade, reducing evaporation water consumption caused by bare soil in the tea garden, and improving the humidity of the microenvironment of the plantation. This is conducive to the coverage of large, relatively uniform, and continuous distribution of legumes in the inner vascular plant community. It has promoted the colonization of rhizobia in the soil of the plantation (Zhang et al., 2011), significantly increased the total amount of microorganisms in the soil of the interplanting of pecan and tea trees, and enriched beneficial bacterial genera with nitrogen fixation functions, such as*Allorhizobium* , *Neorhizobium* ,

Pararhizobium ,*Rhizobium* (Figs. 1a and 1d). In this study, the significant increase in soil NO_3-N may be caused by the enhancement of nitrogen fixation and the weakening of denitrification. There was a significant positive correlation between NO_3-N and microbial community richness (Chao 1 index and observed_spieces richness) and the relative abundance of*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (Figure 2), and random forest analysis showed that Chloroflexi and Firmicutes were relatively enriched in pure tea garden soils (Figure 6). Chloroflexi and Firmicutes are the main participating species in the nitrification and denitrification process of microorganisms (Calderoli et al., 2018; Fernández et al., 2009), indicating that pecan tea tree lasses can effectively make the soil nutrient of the plant available and improve the material recycling capacity of the tea rhizosphere microecosystem (Chen et al., 2017).

Based on the analysis of phyla classification level, this study found that under the two planting modes, Proteobacteria, Actinobacteria, and Acidobacteria were the dominant bacteria in the plantation soil, but the proportions of Proteobacteria and *Actinomyces* increased in the soil of walnut tea interplanting, while the proportions of Acidobacteria decreased. Proteobacteria as a major group of bacteria includes a variety of functional bacteria, such as *Allorhizobium* ,*Neorhizobium* , *Parahizobium* , and *Rhizobium* with nitrogen fixation function and *Enterobacter* with pollution degradation function (Jones et al., 2009).

Mendes et al. (Mendes et al., 2011) believed that the relative abundance of Actinobacteria was positively related to disease resistance, and some of them could secrete antibiotics to inhibit the propagation of pathogenic bacteria; Actinobacteria was one of the most active groups related to disease inhibition. At the same time, previous studies have found that *Pedosphaeraceae* may be a key group with an important ecological function metabolic pathway, which has important potential in promoting plant growth and tolerance to heavy metal biotoxicity (Yuan et al., 2022; Chun et al., 2021). In this study,*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and*Enterobacteriaceae* among Proteobacteria, *Coriobacteriaceae_UCG-002* of Actinobacteria, and*Pedosphaeracea* were significantly enriched in the soil of tea garden interplanted with pecan, indicating that pecan-tea interplanting have growth-promoting effects on soil disease inhibiting bacteria and probiotic bacteria. This effectively protects crops from infection by soil-borne pathogens and promotes plant growth by rebalancing the microbial community structure of the soil.

The pecan-tea interplanting favor complex interactions of soil microbial communities (Figure 1a). Consistent with a previous study of woody-tea interplanting (Jiao et al., 2016), the results of this study showed that pecan-tea interplanting could significantly enhance the soil microbial community composition of the alpha diversity of the plantations (Figure 1b). In addition to increasing potential interactions among different microbial populations, the promoting effects of pecan-tea interplanting accessions on soil microbial growth and activity may result in increased links between functional modules and increased complexity of microbial community co-occurrence networks (Figure 1a; Table 1).

In the co-occurrence network diagram, microorganisms at many nodes in module 1 were involved in the electron transfer process, most taxa in module 2 were involved in the carbon and nitrogen cycle process of biogeochemistry, most microorganisms in module 4 and module 0 were involved in the degradation process of organic pollutants, and the co-occurrence pattern was nonrandom and driven by microbial functions (Table. 1). Therefore, pecan-tea interplanting is beneficial for maintaining the complex biological processes present in the microbial community.

Nodes in different modules may serve different functions and there are strong ecological links, with a decreased ratio, which is mutually exclusive to co-occurrence in the co-occurrence network, suggesting a low degree of microbial mutual exclusivity in soil under the pecan-tea interplanting (Figure 1a and Table 1) (Wang et al., 2022), possibly because pecan root systems are widely distributed radially. The potential co-existing relationship between microorganisms is protected and maintained by exuding more organic matter through a broad and dense root system, which is beneficial to maintain the stability of the rhizosphere microbiota.

Carya illinoinensis provides a large amount of litter and root exudates for the soil in a tea garden. The plant secondary metabolites in the litter are released into the soil along with the decomposition process, affecting the physical and chemical properties of the soil and the diversity of microbial communities. The decomposition

of litter is the core process of nutrient cycling in the plantation ecosystem, and soil microorganisms are one of the key factors determining the decomposition rate (Zhang et al., 2004). Previous studies have found that root exudates play a selective role in shaping rhizosphere microbial community structure (Wu et al., 2014). The rhizosphere microbial community structure of different plants is unique and representative. The type of root exudates determines rhizosphere microbial community structure and disease suppression function (Gu et al., 2020; Paterson et al., 2007). Conversely, changes in rhizosphere microbial community structure also have an important effect on plant root exudate release, soil material circulation, energy flow, and information transmission. It further affects the growth and development of plants (Esenhauer et al., 2012); thus, there may be a coevolutionary relationship between plants and soil microorganisms. Interplanting pecan in a tea garden can effectively improve the structure of the soil microbial community in the rhizosphere of tea trees, which is conducive to maintaining the balance of the microecosystem in the garden and promoting the growth and stress resistance of tea trees.

After verifying the reliability of the OPLS-DA model and there were no outliers and overfitting, the VIP method was used to screen out the tea differential quality indicators under different planting modes. Combined with PCA analysis, the comprehensive score of tea samples was obtained. The results showed that the interplanting of pecan and tea significantly improved the comprehensive quality of tea leaves (Figure 4c). The results of the correlation analysis indicate that the amino acids of tea with VIP value of >1.0 are all related to the alpha diversity (Chao 1 and observed species richness indicators) except for Gly, GABA, and Asp. However, Gly, GABA, and Asp are significantly and positively correlated with *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* ,*Enterobacter* , and*Coriobacteriaceae_UCG-002* , which are enriched in the soil of walnut tea interplanting (Figure 5). This indicates that walnut tea interplanting will transform the soil microbial community structure to be more conducive to the formation of tea quality.

Previous studies have found that the heat distribution of interplanted tea plantations tends to be balanced compared with that of pure tea plantations, and proper shading and reduction in direct radiation are conducive to regulating carbon and nitrogen metabolism in tea plants. Under shading conditions, the nitrogen content of tea leaves increases, and the total trend of carbon compounds content decreases (Zhou et al., 1995), which is consistent with the increase in theobromine and part of the free amino acid content of tea leaves in the interplanted tea plantations in this study. The results of the reduction in tea polyphenol content are consistent (Table 2), which may be because these nitrogen compounds can absorb blue-violet light, especially the aromatic amino acids, that affect the freshness and aroma of tea. Owing to the large total evapotranspiration of the interplanted tea garden, the turbulent exchange of weak water vapor is not easy to escape, which is conducive to the increase in the water content of the leaves (Wang et al., 2014). The interplanted tea garden has a large proportion of scattered radiation, which promotes the absorption of blue-violet light by the tea leaves, and improves the chlorophyll content and green retention of the leaves (Table 2), making the tea in the interplanted tea garden superior to the monoculture in terms of shape, leaf, and bottom.

5 | CONCLUSIONS

We investigated the effects of walnut tea interplanting plantation on soil properties, microbial community structure, and tea quality. In the planting garden interplanted with pecan and tea trees, the total number of microorganisms in the rhizosphere of tea trees increased, and the*Allorhizobium* , *Neorhizobium* , *Pararhizobium* , and*Rhizobium* species with nitrogen fixation function were enriched. In addition, the relative abundance of Chloroflexi and Firmicutes, which dominated the denitrification and denitrification process, decreased, and the nitrogen fixation function of the microbial colony was enhanced, denitrification was weakened, the soil NO_3-N content increased, and the nutrient availability was improved. Moreover, the interplanting of pecan and tea tree made probiotics, such as*Enterobacter* and *Pedosphaeraceae* , which degrade pollution, and Actinomycetes, which inhibit soil-borne diseases, accumulate in the rhizosphere soil, reduce the mutual exclusion of the microbial community symbiotic network, increase complexity, and stabilize the interaction between microorganisms. The proper shade provided by the pecan can regulate the energy distribution in the plantation and the microclimate under the forest so that the carbon and nitrogen metabolism in the tea

tree is more conducive to the formation of tea quality. Therefore, walnut tea interplanting can significantly improve the comprehensive quality of tea through the transformation of soil microbial community structure and understory environment. In addition, the interplanting of pecan and tea tree not only makes full use of the three-dimensional space of the plantation, but also realizes "multiple use of one land" and "multiple harvest in one year." Reducing industrial risk has significant economic and ecological benefits, which can be promoted and applied as a direction of agricultural structure adjustment.

DATA AVAILABILITY STATEMENT

Transcriptome raw reads sequence data are available are available through the NCBI[?]Sequence[?]Read[?]Archive (BioProject:PRJNA905202).

AUTHOR CONTRIBUTIONS

We would like to thank Shuiping Yang for their suggestions and comments on the manuscript. J.C. designed the study. Q.H., X.Y. and H.R. collected samples. J.C. and S.W. performed the data processes. S.W. wrote the manuscript. All authors read and approved the final manuscript.

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Figures

FIGURE 1 Effects of different intercropping measures on the microbial community structure and diversity of tea soil.

A Network of soil microbial populations in tea soil. The red lines indicate positive correlations and the green lines indicate negative correlations. The area of the node is proportional to the node degree, calculated from

correlations of abundances for each ASV (Identity=100%). Only correlations with an r of > 0.6 or $<$ -0.6 and a P- value of < 0.05 were included in the network.

B Alpha diversity values for soil microbial communities under different intercropping measures.

C NMDS analysis of soil community compositional variation based on Bray-Curtis distances.

D Random forest classification analysis of the dominant bacteria in plantations of different interplanting measures.

FIGURE 2 Correlation between soil properties and soil microbiome structures and diversities.

A Correlations between soil physicochemical parameters and alpha diversity index values.

B Correlations between the abundances of the dominant bacteria identified using random forest classification as discriminatory for sample groups with tea soil physicochemical properties.

FIGURE 3 Genus differences of the microbiota of the two groups were compared by wilcox.test. (**a.** The ordinate represents the difference species, while the abscissa represents the mean value of the genus abundance. **b.** The difference of the abundance between the x-coordinate groups, the color of the points represents the group with higher abundance, the error bar of the points represents the fluctuation range of the 95% confidence interval of the difference, and the y coordinate represents the significance of the difference between the corresponding species groups.)

FIGURE 4 Effects of different intercropping measures on microbial community structure and diversity of tea soil.

A OPLS-DA score map of tea quality components.

B VIP value histogram of tea evaluation index.

C A comprehensive evaluation score box chart was obtained via factor analysis based on the tea quality index with a VIP value of > 1.0.

FIGURE 5 Correlation between tea quality indexs and soil microbiome structures and diversities.

A Correlations between alpha diversity index values and tea quality index with VIP value of > 1.0.

B Correlations between the abundances of dominant bacteria identified using random forest classification as discriminatory for sample groups with tea quality index with VIP value of > 1.0.

FIGURE 6 Top 10 bacterial species were identified at the phylum taxonomic level by applying random-forest classification of the relative abundance of the tea rhizosphere microbiota. (Biomarker taxa are ranked in descending order of importance to the accuracy of the model. The 4 inset represents ten-fold cross-validation error as a function of the number of input families used 5 to differentiate CK groups and T groups in order of variable importance.)

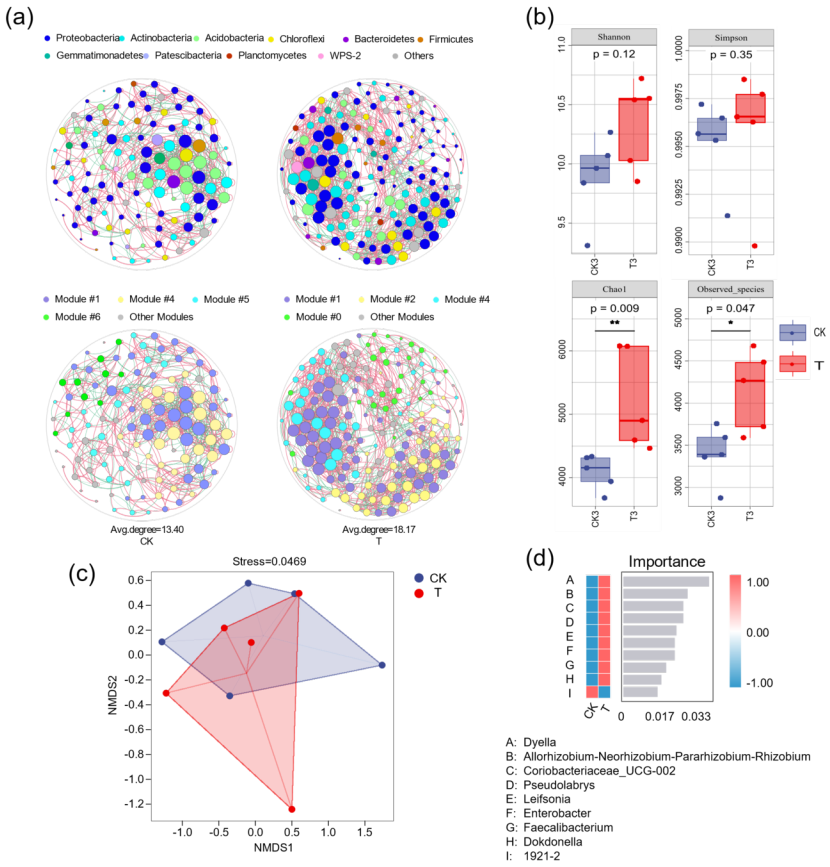

Figure 1

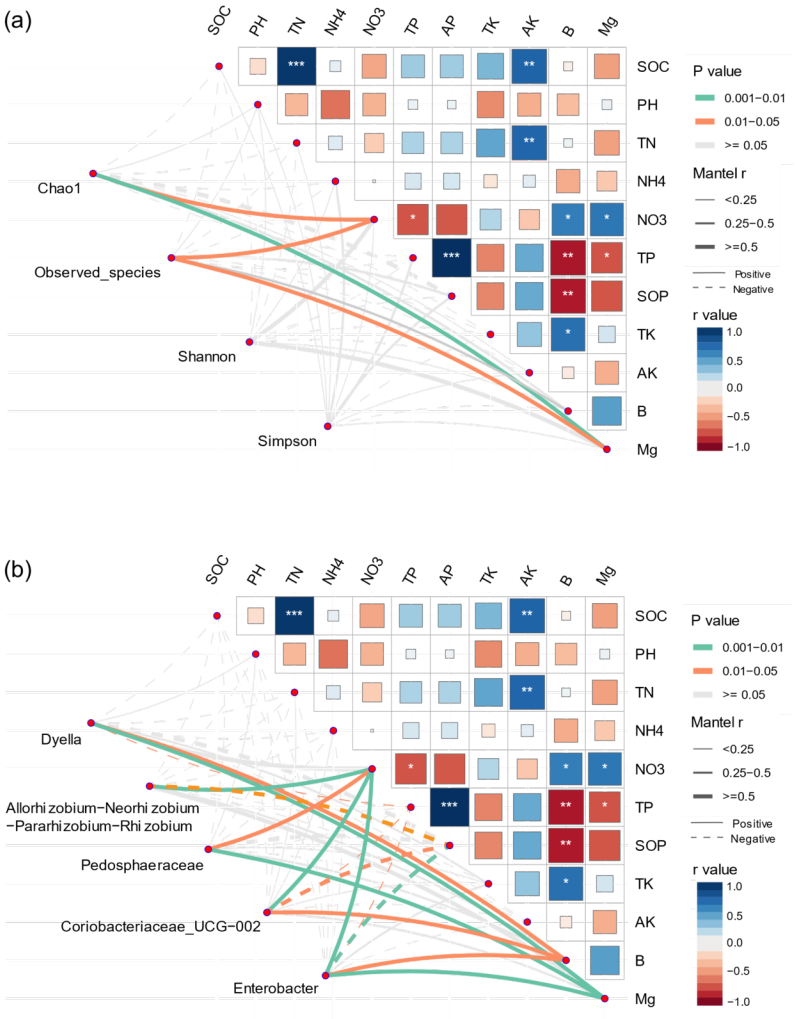

Figure 2

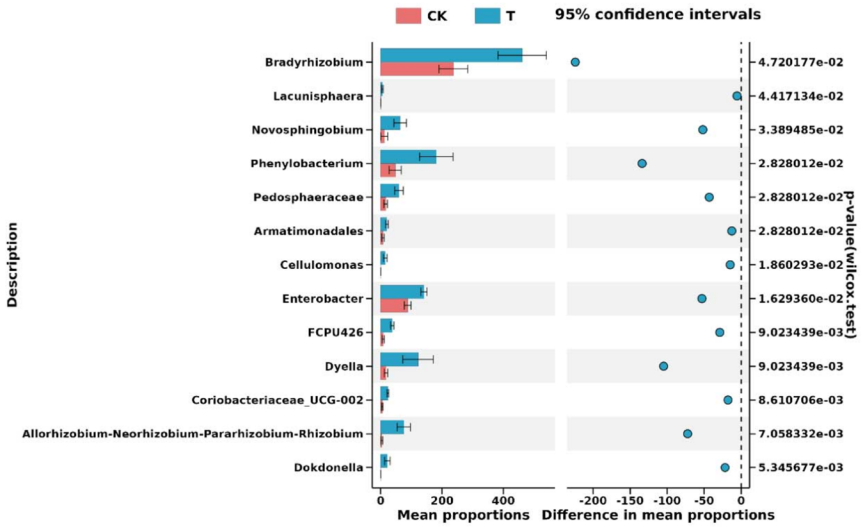

Figure 3

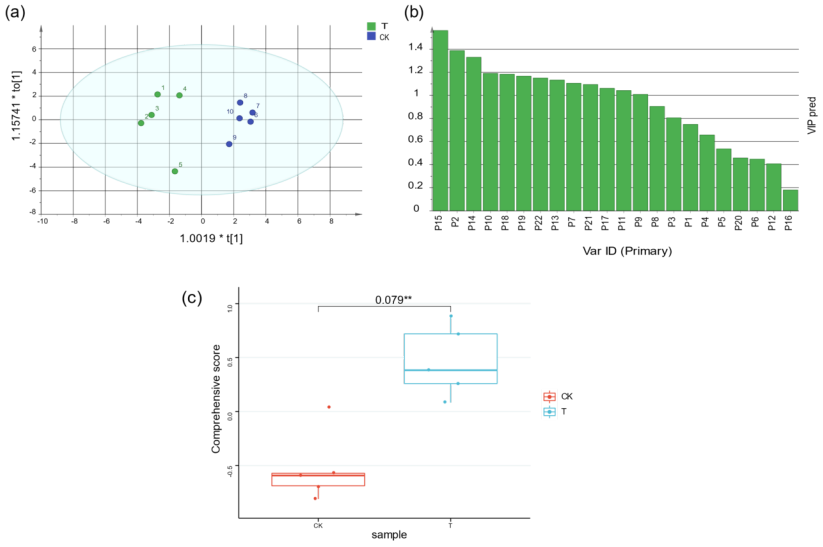

Figure 4

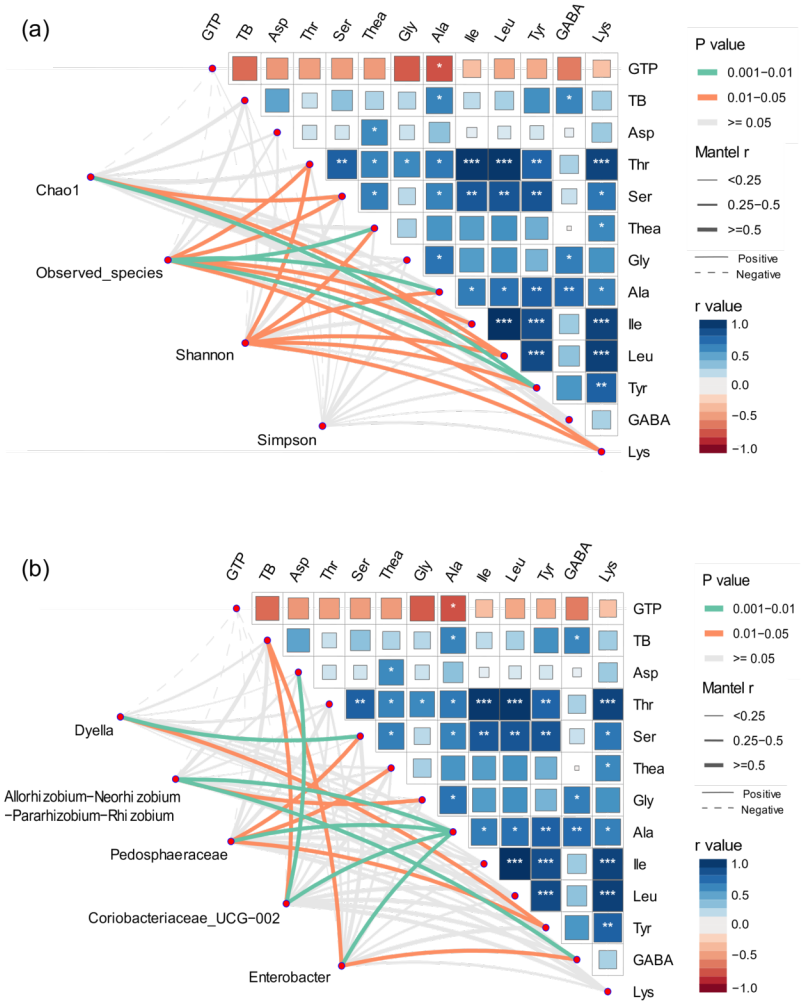

Figure 5

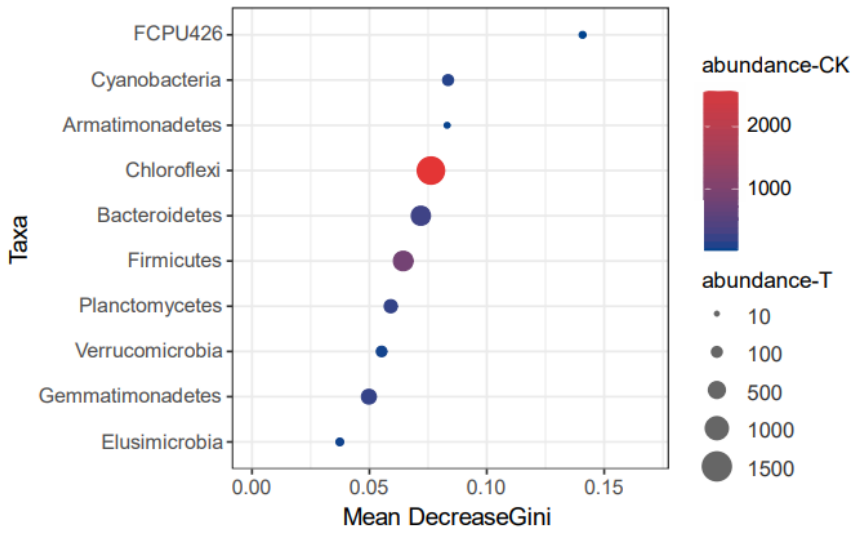

Figure 6

Table

TABLE 1 Bacterial co-occurrence network characteristics of pecan.

TABLE 2 Quality characteristics of tea in tea gardens with different interplanting measures

TABLE 1

Treatment	Node	Positive edge	Negative edge	Avg.degree	Modularity[a]	Avg.clustering coefficient[b]	Avg.path dis
CK	117	459	323	13.402	9.968	0.683	3.501
T	166	930	578	18.169	3.399	0.638	3.266

[a] Degree of nodes tending to differentiate into different network modules.

[b] Degree of nodes tending to cluster together.

[c] Network path distance is the length of the shortest path between two nodes within the network.

TABLE 2

Sample	Chl	GTP	C	C	EC	EC	GA	GC	EGC	ECG	ECG	GCG	GCG	EGCG
CK1	1.15	3.82	-	-	0.026	0.026	0.048	-	-	0.017	0.017	-	-	0.125
CK2	0.82	4.45	-	-	0.015	0.015	0.049	-	-	0.019	0.019	-	-	0.067
CK3	0.86	4.03	-	-	0.075	0.075	0.044	-	-	0.016	0.016	-	-	0.034
CK4	0.90	3.85	-	-	0.067	0.067	0.040	-	-	0.015	0.015	-	-	0.026
CK5	0.78	4.08	-	-	0.018	0.018	0.066	-	-	0.016	0.016	-	-	0.078
T1	1.09	3.66	-	-	0.006	0.006	0.026	-	-	0.030	0.030	-	-	0.031
T2	1.01	3.40	-	-	0.004	0.004	0.030	-	-	0.028	0.028	-	-	0.036
T3	1.03	3.54	-	-	0.004	0.004	0.045	-	-	0.012	0.012	-	-	0.034
T4	0.85	3.69	-	-	0.067	0.067	0.045	-	-	0.011	0.011	-	-	0.040
Sample	TB	THEO	Caf	Caf	Asp	Asp	Thr	Ser	Glu	Thea	Thea	Gly	Gly	Ala
CK1	0.019	-	0.736	0.736	0.025	0.025	0.026	0.068	0.273	0.817	0.817	0.004	0.004	0.047
CK2	0.022	-	0.756	0.756	0.023	0.023	0.025	0.06	0.238	0.781	0.781	0.005	0.005	0.069

Sample	Chl	GTP	C	C	EC	EC	GA	GC	EGC	ECG	ECG	GCG	GCG	EGCG
CK3	0.031	-	0.709	0.709	0.039	0.039	0.027	0.072	0.342	1.024	1.024	0.004	0.004	0.045
CK4	0.045	-	0.709	0.709	0.087	0.087	0.023	0.064	0.32	1.045	1.045	0.005	0.005	0.091
CK5	0.032	-	0.749	0.749	0.029	0.029	0.042	0.119	0.447	1.023	1.023	0.006	0.006	0.101
T1	0.028	-	0.775	0.775	0.144	0.144	0.045	0.117	0.289	1.760	1.760	0.012	0.012	0.210
T2	0.043	-	0.834	0.834	0.037	0.037	0.038	0.089	0.221	0.897	0.897	0.016	0.016	0.246
T3	0.054	-	0.773	0.773	0.181	0.181	0.032	0.112	0.491	1.260	1.260	0.006	0.006	0.188
T4	0.045	-	0.72	0.72	0.026	0.026	0.041	0.159	0.621	1.394	1.394	0.007	0.007	0.261
T5	0.050	-	0.734	0.734	0.121	0.121	0.038	0.099	0.358	1.245	1.245	0.010	0.010	0.282
Sample	Val	Cys	Met	Ile	Ile	Leu	Tyr	Phe	GABA	Lys	His	His	Arg	Pro
CK1	0.028	-	-	0.007	0.007	0.011	0.026	0.032	0.058	0.006	-	-	-	-
CK2	0.026	-	-	0.009	0.009	0.014	0.042	0.024	0.087	0.011	-	-	-	-
CK3	0.021	-	-	0.008	0.008	0.013	0.031	0.039	0.05	0.010	-	-	-	-
CK4	0.033	-	-	0.006	0.006	0.012	0.043	0.042	0.114	0.010	-	-	-	-
CK5	0.026	-	-	0.019	0.019	0.029	0.082	0.039	0.082	0.022	-	-	-	-
T1	0.034	-	-	0.017	0.017	0.028	0.062	0.023	0.053	0.022	-	-	-	-
T2	0.011	-	-	0.015	0.015	0.025	0.071	0.036	0.354	0.017	-	-	-	-
T3	0.036	-	-	0.012	0.012	0.02	0.069	0.036	0.149	0.016	-	-	-	-
T4	0.030	-	-	0.018	0.018	0.029	0.098	0.054	0.200	0.017	-	-	-	-
T5	0.031	-	-	0.015	0.015	0.024	0.082	0.058	0.311	0.019	-	-	-	-

*Chl: chlorophyll, mg/g; GTP: tea polyphenols (%); C: catechin (%); EC: epicatechin (%); GA: gallic acid (%); GC: gallic catechin (%); EGC: epigallocatechin (%); CG: catechin gallate (%); ECG: epicatechin gallate (%); GCG: epigallocatechin gallate (%); EGCG: epigallocatechin gallate (%); TB: theobromine (%); THEO: theophylline (%); Caf: caffeine (%); Asp: aspartic acid (%); Thr: threonine (%); Ser: serine (%); Glu: glutamic acid (%); Thea: theanine (%); Gly: glycine (%); Ala: alanine (%); Vla: valine (%); Cys: cystine (%); Met: methionine (%); Ile: isoleucine (%); Leu: leucine (%); Tyr: tyrosine (%); Phe: phenylalanine (%); GABA: gamma aminobutyric acid (%); Lys: lysine (%); His: histidine (%); Arg: arginine (%); Pro: proline (%). "-" indicates that the substance is not detected under the current method accuracy.