

Article

Changes in Rhizosphere and Bulk Soil Microbial Communities of Tableland Tea Garden and Ancient Tea Plantation in Southwest China

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Abstract: Tea (*Camellia sinensis* L.), an important economic crop in China, is highly favored by the population. Microorganisms can help plants acquire soil nutrients and cope with various stresses, and the diversity and structural composition of the rhizosphere microbial community of tea plants are crucial for ensuring the growth and quality of tea leaves. Therefore, we studied the differences in soil nutrients, enzyme activities and microbial communities between two different tea gardens (a tableland tea garden and an ancient tea plantation) in different ecological niches (rhizosphere and bulk soil), as well as the impacts they experienced. The results show that the soil pH levels in the ancient tea plantation were within the optimal range (4.5–5.5), and both rhizosphere and bulk soil nutrients in the ancient tea plantation were higher than those in the tableland tea garden, except for TP; the nutrients in the rhizospheres of ancient tea trees were more abundant. Moreover, higher enzyme activities were observed in the rhizosphere soil than those in the bulk soil in both tea gardens, and both the tableland and ancient tea garden soils were subjected to a certain degree of C&N limitations. The microbial communities of the two tea gardens were dominated by bacteria, but the α -diversity of the bacterial and fungal communities in the rhizosphere soil of the tableland tea garden was higher than that in the ancient tea plantation. The bacterial communities were largely dominated by *Proteobacteria* and *Acidobacteriota*, and the fungal communities were largely dominated by Ascomycota and Basidiomycota in the two tea gardens. The structure and composition of soil bacterial communities in the two tea gardens were similar, whereas significant differences were observed in the fungal communities. In addition, soil pH and SWC were the key factors influencing the fungal community in both the rhizosphere and bulk soil in the two tea gardens, whereas the bacterial community was more significantly affected by soil TN, $\text{NH}_4^+\text{-N}$, SWC and DON. These findings provide essential foundational information for the preservation of ancient tea plantations, the ecological adaptability of ancient tea trees and the management of tableland tea gardens.

Keywords: microbial community; rhizosphere and bulk soil; tableland tea garden; ancient tea plantation



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1. Introduction

Yunnan Province in China is one of the global centers of origin for tea plants, currently preserving the largest number of ancient tea trees [1]. Qianjiazhai Nature Reserve of Ailao Mountain in Yunnan Province is the largest and best-preserved wild ancient tea plant community [2,3]. Among them, the wild tea species of *Camellia talinensis* has a long history of cultivation, domestication and consumption. After extensive cultivation by local people and natural screening, ancient tea has nurtured abundant tea types, resources and

many excellent species, with a long history of local tea culture and rich biodiversity [1,4]. Ancient tea trees provide substantial economic, cultural, scientific and landscape values. However, they have been subjected to various degrees of destruction, including habitat destruction, predatory harvesting, mechanical exploitation, indiscriminate felling and a lack of management practices. In addition, imbalances in the soil ecosystem also put ancient tea trees at risk of population decline. Good soil conditions are fundamental for the healthy growth of tea trees and the production of high-quality tea leaves. In order for ancient tea tree resources to sustain long-term development, the protection of their rich diversity and the scientific and rational utilization of these resources are urgently needed. Therefore, the analysis of the soil ecosystem of ancient tea trees is of critical importance for conserving ancient tea trees, improving soil conditions, and the enhancement of tea quality.

Soil microbial communities, comprising bacteria and fungi, play a vital role in ecological processes such as nutrient cycling, the formation of soil organic matter and the establishment and stabilization of soil structure [5,6]. The rhizosphere is a complex region of interaction between plants and soil, where plant roots provide abundant nutrients and energy for microorganisms, thereby significantly influencing the physical, chemical and biological properties of the soil [7,8]. Rhizosphere soil typically exhibits higher microbial and enzyme activities, whereas these characteristics are relatively lower in bulk soil [9]. In recent years, soil microbial communities associated with tea trees have received increasing attention, and the beneficial role of microorganisms in enhancing nutrient uptake, promoting growth and improving the quality of tea leaves has been recognized [10,11]. In turn, rhizospheric microorganisms are subjected to various influences from their host plants, such as root exudates, species, genotypes and developmental stages, while concurrently obtaining the necessary nutrients for growth from these hosts [12,13]. Rhizodeposits, composed of sloughed-off tissue cells and root exudates, are rich in carbon and nitrogen, which are beneficial to the growth of rhizosphere microorganisms [14]. However, rhizodeposits are essentially subject to the control of different hosts [10]. For instance, tea plants selectively shape the assembly of the rhizosphere microbiome by exuding L-theanine and regulating the cycling of elements (C, N, P, S) [15]. Moreover, plant health and nutrition are significantly affected by rhizosphere soil [16]. Wang et al. (2020) [17] studied the microbial diversity of rhizosphere soil in Tieguanyin tea trees of different ages and found that, with the increase in the age of tea trees, the number of pathogenic microorganisms in the rhizosphere soil increases, whereas the quantity of microorganisms decomposing harmful substances, probiotics and those involved in carbon and nitrogen cycling decreases. Rhizosphere soil enhances soil nutrient input and aggregate stability, creating a favorable environment for microorganisms and boosting soil enzyme activity to promote soil carbon assimilation [18]. Soil enzymes are important components that catalyze the decomposition and transformation of soil nutrients, and their activity is a vital indicator of soil health, primarily driven by readily available carbon sources provided by root turnover and root exudates [19]. Moreover, the stoichiometry of soil enzymes reflects the relative proportions of different enzymes involved in the cycling of key nutrients such as carbon (C), nitrogen (N) and phosphorus (P) [20], providing insights into soil nutrient demands and microbial processes. Previous studies have shown that arbuscular mycorrhizal fungi enhance plant growth by establishing a mutually beneficial symbiotic relationship with plant roots, thereby increasing soil enzyme activity, which constitutes a positive symbiotic interaction [21]. Hence, different rhizosphere environments have been shaped by different microbial communities, with each community having a different impact on soil nutrient cycling and with microbial communities playing a crucial role in these processes.

In this study, we investigated the differences in bacterial and fungal communities between rhizosphere soil and bulk soil in tableland tea gardens and ancient tea plantations, as well as their main influencing factors. We hypothesized that (1) the physicochemical properties, enzyme activities and microbial communities of the two tea gardens are significantly influenced by the rhizosphere environment; (2) changes in different tea ages in the tableland tea garden and ancient tea plantation, including variations in the tea garden

litter, anthropogenic disturbances and tree size, cause diverse impacts on the bacterial and fungal communities in the rhizosphere and bulk soil of the two tea gardens; and (3) microbial communities are directly influenced by the physicochemical properties in tea garden soils. Therefore, the main objectives of our study were to (1) investigate the variations in soil physicochemical properties, enzyme activities and microbial communities in the rhizosphere and bulk soil of the two tea gardens; (2) explore whether changes in soil physicochemical properties and enzyme activities have different impacts on bacterial and fungal community structures; and (3) determine the primary driving factor in microbial community structure and diversity in the tableland tea garden and ancient tea plantation.

2. Materials and Methods

2.1. Experiment Site and Sampling

The experimental sampling was conducted in the spring (April) of 2021 at Jiu Jia Town (101°13' E, 24°12' N) in Zhenyuan County, Pu'er City, China (outside the Qianjiazhai Nature Reserve). The region is characterized by a complex topography with an alternating landscape of mountains and rivers, contributing to a pronounced vertical climatic gradient. The area has an average annual temperature of 17 °C, receives between 1650 and 1750 mm of precipitation annually and has a mean frost-free period of 330 days per year. In the surroundings of the village, there are extensive ancient tea plantations formed by ancient tea trees domesticated by predecessors, as well as artificially managed tableland tea gardens. In the tableland tea gardens, harvesting occurs 2–4 times in spring, summer and autumn, respectively, followed by plowing, fertilizing, pruning and pesticide application in winter before the gardens are closed. The ancient tea plantations are harvested only once in the spring and undergo minimal pruning, reflecting a more extensive management style. Both tea gardens plant *Camellia talinensis*. The basic situation of the tea gardens is detailed in Table S1.

To achieve comprehensive coverage of the entire tea plantation with sampling points, three sampling points were established along the distribution belt of the tea trees. At each sampling site, three tea plants were randomly selected for soil sample collection, which were then mixed into a single sample, maintaining a minimum distance of 15 m from any other tea plants [8,22]. During the sampling process, soil blocks with intact root systems (5–20 cm in depth) were excavated. Loosely bound soil was shaken off, and soil adhering to the roots was collected and defined as rhizosphere soil. Bulk soil was collected from soil within a 10 m radius of the tea plants, where there were no tea trees or other plant roots present [23], at the same depth of 5–20 cm. The rhizosphere and bulk soil samples from each sampling site were mixed separately, which were then promptly sieved on-site to remove impurities such as stones and root debris and minimize the influence of microbial activities [19]. A total of 12 composite soil samples were obtained and transported to the laboratory in ice boxes for subsequent analysis. Soil samples were subdivided into three portions: one was air-dried for the analysis of soil nutrients, another was stored at −20 °C for the determination of soil enzyme activities, and the final portion was preserved at −80 °C in an ultra-low temperature freezer for the analysis of soil microbial communities.

2.2. Soil Property Analyses

Soil water content (SWC) was determined by the gravimetric method. Soil pH was measured with a pH meter using a soil:water ratio of 1:2.5 (*w/v*). Total carbon (TC) and total nitrogen (TN) were quantified using an elemental analyzer (Vario MAX, Elementar, Langenselbold, Germany), and total phosphorus (TP) was assessed by the molybdenum–antimony colorimetric method. Soil nitrate nitrogen (NO_3^- -N) and ammonium nitrogen (NH_4^+ -N) were assessed using the ultraviolet spectrophotometric method (phenol disulfonic acid colorimetry) and the KCl extraction–indophenol blue colorimetric method, respectively. Dissolved organic C (DOC) and total soluble N (TSN) were measured by a TOC-VCPH/CPN analyzer (Shimadzu Scientific Instruments, Kyoto, Japan) after extracting

with 2 M KCl using a soil:water ratio of 1:5. Dissolved organic N (DON) was calculated according to the differences between TSN and total inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$).

2.3. Soil Enzyme Activity Assays and Vector Analysis of Enzyme Activities

β -1,4-glucosidase (β G), N-acetyl- β -D-glucosaminidase (NAG), leucine aminopeptidase (LAP), acid phosphatase (ACP), cellobiohydrolase (CBH) and soil polyphenol oxidase (PPO) were capable of oxidatively degrading phenolic compounds in the soil. The activities of these soil extracellular enzymes were all measured using protocols from ELISA (Enzyme-Linked Immunosorbent Assay) (Jiangsu Mei-mian Industrial Co., Ltd., Yancheng, China) research kits.

The C:N, C:P and N:P stoichiometric ratios of soil enzyme activity were expressed as $\ln \text{C}:\ln \text{N} = \ln \beta\text{G}/\ln (\text{NAG} + \text{LAP})$, $\ln \text{C}:\ln \text{P} = \ln \beta\text{G}/\ln \text{ACP}$ and $\ln \text{N}:\ln \text{P} = \ln (\text{NAG} + \text{LAP})/\ln \text{ACP}$ [24], based on log-transformed soil enzyme activity ratios. The vector length was conducted to quantify relative C vs. nutrient limitation, and microbial C limitation increased with the vector length. Vector angles were applied to quantify the relative P vs. N limitation. A vector angle $> 45^\circ$ indicates the relative P limitation of microorganisms, with the opposite interpretation for the relative N limitation. The more a vector angle deviates from 45° , the more severe the relative limit. Vector length and vector angle were calculated according to Formulas (1) and (2) [20]. The scatter plot of $\ln \text{C}:\ln \text{P}$ and $\ln \text{C}:\ln \text{N}$ was also represented as the C&N limitation and C&P limitation based on the deviation from the expected enzyme activity ratios of C:N (1:1) and N:P (1:1) [25].

$$\text{Vector L} = \sqrt{(\ln \text{C} / \ln \text{P})^2 + (\ln \text{C} / \ln \text{N})^2} \quad (1)$$

$$\text{Vector A} = \text{Degrees} [\text{ATAN2} [(\ln \text{C} / \ln \text{C}), (\ln \text{C} / \ln \text{N})]] \quad (2)$$

2.4. DNA Extraction, PCR Amplification and High-Throughput Sequencing

Soil DNA was extracted from the samples using the CTAB method, followed by agarose gel electrophoresis to assess the purity and concentration of the DNA. An appropriate amount of sample DNA was then transferred to a centrifuge tube and diluted to a concentration of 1 ng/ μL with sterile water. Using the diluted genomic DNA as a template, specific barcoded primers were selected based on the sequencing region of interest. PCR amplifications were performed with the Phusion[®] High-Fidelity PCR Master Mix with GC Buffer from New England Biolabs (New England, USA) and a high-efficiency, high-fidelity enzyme to ensure amplification efficiency and accuracy. Primer pairs targeting specific regions included 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for the 16S V4 region to identify bacterial diversity and ITS5-1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3') for the ITS1 region to identify fungal diversity. Additionally, the amplified regions also encompassed 16S V3-V4, 18S V9, and ITS2. The PCR reaction mixture comprised 15 μL of Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each primer and 10 ng of genomic DNA template. The amplification protocol initiated with an initial denaturation step at 98 $^\circ\text{C}$ for 1 min, followed by 30 cycles of denaturation at 98 $^\circ\text{C}$ for 10 s, annealing at 50 $^\circ\text{C}$ for 30 s and extension at 72 $^\circ\text{C}$ for 30 s. The final extension was carried out at 72 $^\circ\text{C}$ for 5 min.

The PCR products were subjected to electrophoresis on a 2% agarose gel for detection. PCR products that passed the quality check were purified using magnetic beads and quantified enzymatically. Equimolar pooling of the PCR products was performed based on their concentrations, followed by thorough mixing. The mixed PCR products were then analyzed again on 2% agarose gel electrophoresis. Target bands were excised, and the products were recovered using the gel extraction kit provided by Qiagen, Hilden, Germany.

Library construction was performed using the TruSeq[®] DNA PCR-Free Sample Preparation Kit by Illumina (San Diego, CA, USA), and the prepared libraries were quantified

using Qubit and quantitative PCR (qPCR). Once the libraries met the quality criteria, sequencing was carried out on the NovaSeq 6000 platform.

2.5. Statistical Analyses

All data were first tested for homogeneity of variance and normality of distribution, and values were log-transformed when needed. Independent-samples *t*-tests were used to analyze the effects of the rhizosphere or bulk soil of the different tea gardens and the rhizosphere and bulk soil of same tea gardens. The above statistical tests were considered significant for $p < 0.05$. Soil microbial community analyses were conducted using Qiime software (version 1.9.1) and R software (version 4.1.2). For 16S, the annotation database was the Silva 138.1 database, whereas for ITS, it was the Unite database. The same operational taxonomic units (OTUs) were clustered by the sequences at a 97% identity threshold. The richness and diversity of the microbial communities were reflected by the Chao1 and Shannon indices of alpha diversity, respectively. Principal coordinate analysis (PCoA) based on the weighted unifracs distance was conducted to illustrate the similarity of species composition structures. Samples with high similarity in community structures tend to cluster together, whereas those with considerable community differences are likely to disperse far apart. LEfSe (LDA Effect Size) analysis [26] was performed using LEfSe software (version 1.0) (setting: LDA Score > 4), enabling the identification of biomarkers with statistically significant differences between groups. The Mantel test was conducted using the “ggcor” package in R for data calculation, with the results visualized by chiplot (<https://www.chiplot.online/> (accessed on 24 December 2023)). The R software package “plsrm” (1000 bootstraps) was used to validate the estimates of the path coefficients and determination coefficients (R^2) in PLS-PM. Functional predictions of bacterial and fungal communities were conducted using PICRUSt (version 1.0) and Tax4Fun (an R package) analyses, respectively. Redundancy analysis (RDA) was conducted using CANOCO version 5 to further elucidate the contribution of rhizospheric and bulk soil physicochemical factors to the microbial communities in tea gardens.

3. Results

3.1. Rhizosphere and Bulk Soil Physicochemical Properties

As shown in Table 1, the soil SWC, pH, TC, TN, DOC, DON, NO_3^- -N and NH_4^+ -N in the rhizosphere and bulk soil of the ancient tea plantation were significantly higher than those in the tableland tea garden ($p < 0.05$). But, soil TP in both the rhizosphere and bulk soil of the ancient tea plantation was significantly lower compared to that of the tableland tea garden ($p < 0.05$). Moreover, marked differences were observed in soil SWC, pH, TC, TN, TP, DOC, DON, NO_3^- -N and NH_4^+ -N between the rhizosphere and bulk soil of the ancient tea plantation ($p < 0.05$), and the values were significantly higher in the rhizosphere soil than those in the bulk soil. Nevertheless, in the tableland tea garden, SWC, TN and DOC in the rhizosphere soils were higher than those in the bulk soil ($p < 0.05$), whereas the rhizosphere soil pH, TC and TP were significantly lower than those in the bulk soil ($p < 0.05$). There was no significant difference in soil DON, NO_3^- -N or NH_4^+ -N between the rhizosphere and bulk soils in the tableland tea garden.

Table 1. Soil physical and chemical characteristics in the rhizosphere and bulk soil of tableland tea garden and ancient tea plantation.

Sample	Location	SWC (%)	pH	TC (g·kg ⁻¹)	TN (g·kg ⁻¹)	TP (g·kg ⁻¹)	DOC (mg·kg ⁻¹)	DON (mg·kg ⁻¹)	NO ₃ ⁻ -N(mg·kg ⁻¹)	NH ₄ ⁺ -N(mg·kg ⁻¹)
TTG	Rhizosphere	27.11 ± 0.31 Ba	3.66 ± 0.00 Bb	37.95 ± 0.06 Bb	4.08 ± 0.01 Aa	1.18 ± 0.00 Ab	178.10 ± 0.47 Ba	29.12 ± 2.30 Ba	86.83 ± 1.47 Ba	31.53 ± 0.68 Ba
	Bulk	22.60 ± 0.72 Bb	4.10 ± 0.00 Ba	40.84 ± 0.05 Ba	3.47 ± 0.01 Bb	1.21 ± 0.01 Aa	172.30 ± 0.34 Bb	34.18 ± 0.36 Ba	88.33 ± 3.14 Ba	31.92 ± 0.47 Ba
ATP	Rhizosphere	34.22 ± 0.13 Aa	4.60 ± 0.00 Aa	51.59 ± 0.11 Aa	4.05 ± 0.03 Aa	1.02 ± 0.01 Ba	237.79 ± 1.02 Aa	43.20 ± 0.23 Aa	110.73 ± 1.39 Aa	39.91 ± 0.26 Aa
	Bulk	27.30 ± 0.56 Ab	4.57 ± 0.01 Ab	46.05 ± 0.13 Ab	3.62 ± 0.02 Ab	0.92 ± 0.01 Bb	190.71 ± 0.87 Ab	36.45 ± 0.33 Ab	101.51 ± 1.36 Ab	36.86 ± 0.60 Ab

Note: Values are means ± standard deviation (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among different tea gardens within the rhizosphere soil or bulk soil based on independent-samples *t*-tests; different small letters indicate significant differences ($p < 0.05$) among the same tea gardens within the rhizosphere soil and bulk soil based on independent-samples *t*-tests. Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation. Soil property abbreviations are as follows: SWC: soil water content; pH: soil pH value; TC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃⁻-N: nitrate nitrogen.

3.2. Rhizosphere and Bulk Soil Enzyme Activities

The enzyme activities of β G, LAP, CBH and PPO in the rhizosphere soil of the ancient tea plantation were significantly higher than those in the tableland tea garden ($p < 0.05$). The ACP activities in the rhizosphere soil did not show a significant difference between the two tea gardens, whereas the activity of NAG was significantly higher in the tableland tea garden compared to that in the ancient tea plantation ($p < 0.05$). In bulk soils, the enzyme activities of NAG, LAP and CBH were significantly higher in the tableland tea garden compared to those in the ancient tea plantation, whereas the activities of β G, ACP and PPO were significantly lower. The levels of soil NAG, β G, LAP, ACP and PPO were significantly higher in the rhizosphere compared to those in bulk soil ($p < 0.05$), whereas the activity of the CBH enzyme showed no significant difference between the rhizosphere and bulk soil. In the rhizosphere soil of the ancient tea plantation, the activities of β G, LAP and CBH enzymes were significantly higher than those in bulk soil, whereas no significant differences were observed in the activities of NAG, ACP and PPO between the rhizosphere and bulk soil (Table S2).

In comparison to the bulk soil, microbial C limitation in the rhizosphere of the tableland tea garden was significantly higher ($p < 0.05$), but there was no significant difference in the rhizosphere and bulk soil of the ancient tea plantation (Figure 1A). The vector angles in the rhizosphere soil of both the tableland tea garden and ancient tea plantation were $< 45^\circ$, indicating that the N content in the rhizosphere soil mainly limited microbial metabolism, with the microbial N restriction being more severe in the tableland tea garden (Figure 1B). Microorganisms in the rhizosphere soil of both the tableland tea garden and the ancient tea plantation, as well as in the bulk soil of the tableland tea garden, exhibited dual limitations of C&N (vector angle $< 45^\circ$; Figure 1B,C). However, microorganisms in the bulk soil of the ancient tea plantation showed C&P limitations (vector angle $\geq 45^\circ$; Figure 1B,C).

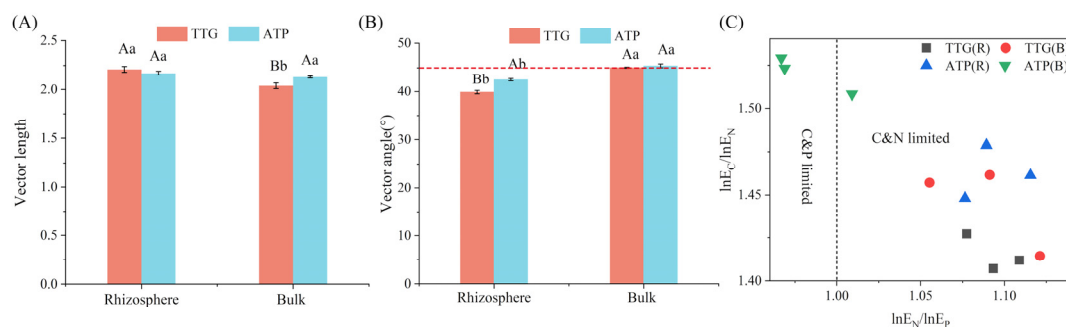


Figure 1. Variation in vector length (A) and vector angle (B) and a scatter plot of soil enzymatic stoichiometry (C). (A,B) Values are means $\pm$ standard deviation ($n = 3$). Different capital letters indicate significant differences ($p < 0.05$) among different tea gardens within the rhizosphere soil or bulk soil based on independent-samples t -tests; different small letters indicate significant differences ($p < 0.05$) among the same tea gardens within the rhizosphere soil and bulk soil based on independent-samples t -tests. (C) $\ln E_C / \ln E_N$ indicates the C:N stoichiometric ratios of the enzymes; $\ln E_N / \ln E_P$ indicates the N:P stoichiometric ratios of enzymes. Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

3.3. Diversity and Composition of Microbial Communities

As shown in Figure S1, the curve tended to be flat, indicating that the amount of sequencing data was progressive and reasonable, and more data can only produce a few new species (OTUs) in the soil samples. In this study, sequences were clustered into 17,690 bacterial OTUs and 5136 fungal OTUs as TTG(R), TTG(B), ATP(R) and ATP(B) (Figure S2).

Significant differences were only observed in the bacterial Chao1 indices of the bulk soil between the tableland tea garden and ancient tea plantation ($p < 0.05$). For the fungal communities, significant differences in soil fungal Shannon indices for rhizosphere soil

between the tableland tea garden and the ancient tea plantation were observed. Furthermore, the fungal alpha diversity between the rhizosphere and bulk soil of the tableland tea garden showed no significant differences. However, in the ancient tea plantation, the fungal alpha diversity was significantly higher in bulk soil than that in rhizosphere soil ($p < 0.05$) (Figure 2).

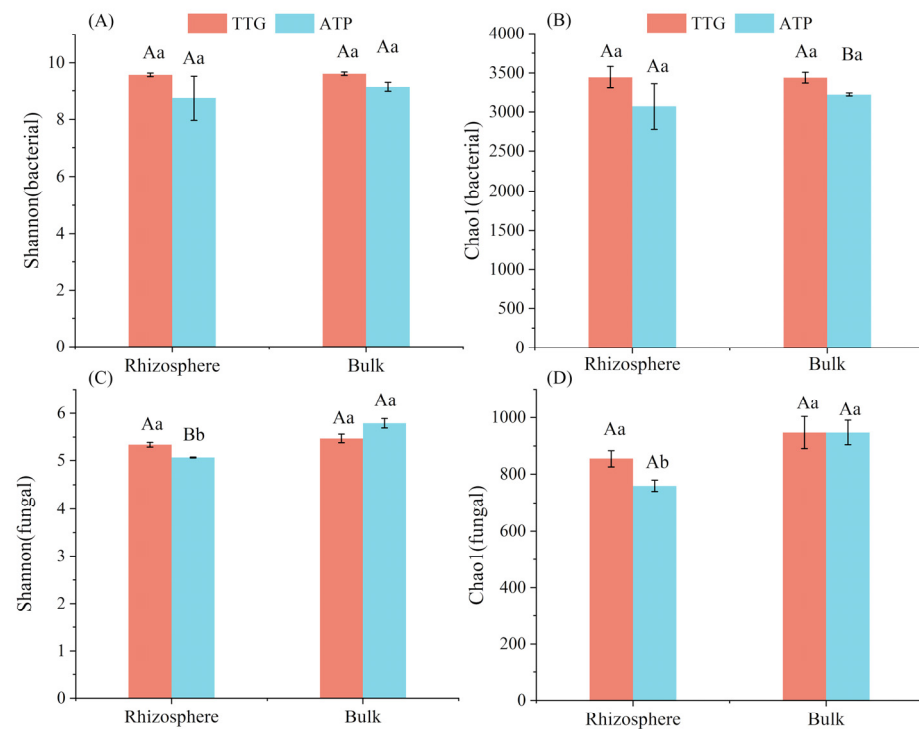


Figure 2. Alpha diversity indices of bacteria (A,B) and fungi (C,D) in the rhizosphere and bulk soil of the tableland tea garden and ancient tea plantation. Different capital letters indicate significant differences ($p < 0.05$) among different tea gardens within the rhizosphere soil or bulk soil based on independent-samples t -tests; different small letters indicate significant differences ($p < 0.05$) among the same tea garden within the rhizosphere soil and bulk soil based on independent-samples t -tests. Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

Overall, the bacterial communities at the phylum level were dominated by *Proteobacteria* (17.9%, 14.4%, 22.6% and 13.3%, respectively), followed by *Acidobacteriota* (21.0%, 24.3%, 19.4% and 28.3%, respectively) and *Firmicutes* (4.2%, 2.9%, 10.1% and 2.1%, respectively) in TTG(R), TTG(B), ATP(R) and ATP(B) (Figure 3A). Fungal communities at the phylum level were dominated by Ascomycota (80.8%, 77.3%, 57.8% and 49.8%, respectively), followed by Basidiomycota (11.8%, 15.1%, 37.0% and 41.5%, respectively) in TTG(R), TTG(B), ATP(R) and ATP(B) (Figure 3B). The relative abundance of *Proteobacteria* and *Firmicutes* was similar in the four soil samples, being higher in the rhizosphere soil compared to the bulk soil in the two gardens. However, the relative abundance of *Acidobacteriota* was greater in bulk soil. Furthermore, the relative abundance of *Proteobacteria* and *Firmicutes* was always higher in ATP(R) than that in TTG(R) (Figure 3A). In comparison with the fungal communities of the ancient tea plantation, the relative abundance of Ascomycota showed significant differences in the tableland tea garden, with the highest in TTG(R) and TTG(B) at 80.8% and 77.3%, respectively. Nevertheless, the relative abundance of Basidiomycota was highest in ATP(R) and ATP(B) at 37.0% and 41.5%, respectively (Figure 3B). The relative abundance of Ascomycota in the rhizosphere soil of the two tea gardens was observed to be higher than that in the bulk soil, but a higher relative abundance of Basidiomycota was detected in bulk soil (Figure 3B).

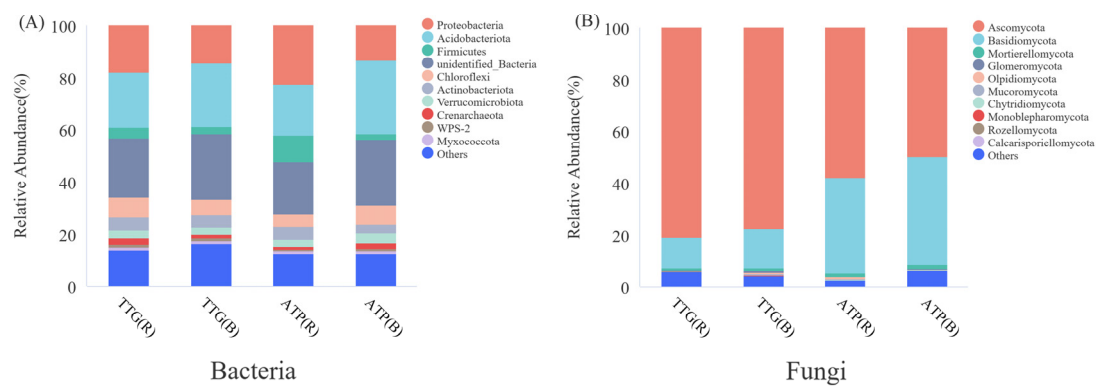


Figure 3. Relative abundance of the top 10 bacteria at the phylum level (A) and the top 10 fungi at the phylum level (B) for TTG(R), TTG(B), ATP(R) and ATP(B). Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

The bacterial communities tended to cluster without obvious separation effects in TTG(R), TTG(B), ATP(R) and ATP(B), indicating that the species of bacterial communities were more similar in composition and structure (Figure 4A). However, the samples were far apart from the fungal communities, and significant difference was found in ATP(R) and ATP(B). The fungal community of TTG(R) was found to overlap with TTG(B) (Figure 4B). According to the linear discriminant analysis effect size (LEfSe) method (Figure S3), for the bacterial communities, the class-level specific species in the rhizosphere soil of the tableland tea garden was Ktedonobacteria, and the phylum-level specific species in the bulk soil of the ancient tea plantation was Acidobacteriota. Moreover, in the two tea gardens, species with significant inter-group abundance variations were more pronounced in fungal communities than those in bacterial communities.

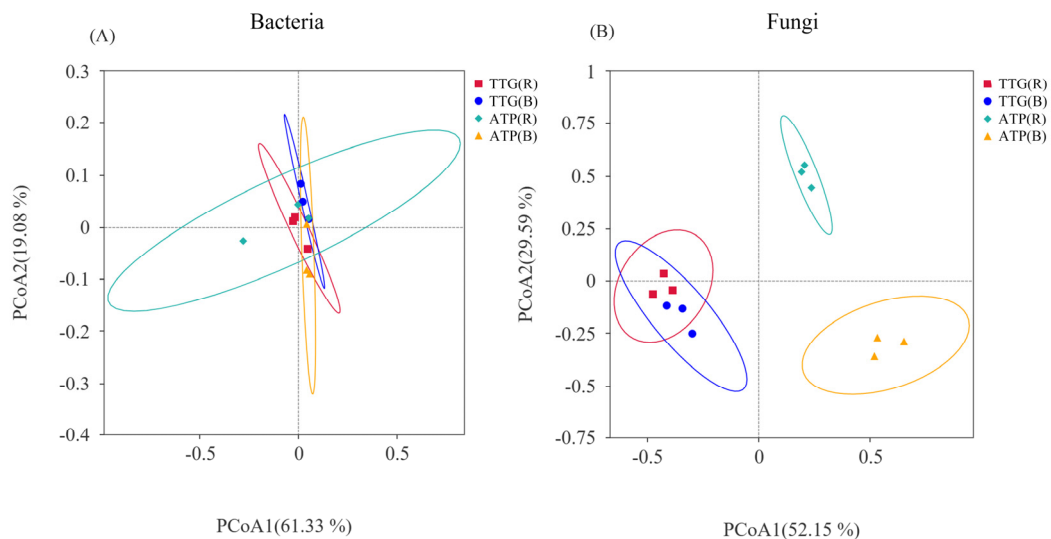


Figure 4. Principal coordinate analysis (PCoA) of soil bacterial (A) and fungal (B) communities based on the weighted_unifrac distance across TTG(R), TTG(B), ATP(R) and ATP(B). Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

3.4. Functional Prediction of Microbial Communities

According to KEGG analysis, six types of biological metabolic pathways (at KEGG 1) were found in TTG(R), TTG(B), ATP(R) and ATP(B) bacterial communities, predominantly consisting of metabolism (51.5%), genetic information processing (16.6%) and environmental information processing (11.5%) (Figure 5A). Among them, compared to ATP(B), TTG(R) and TTG(B), the metabolic pathway of the bacterial community in the ATP(R) group was

significantly down-regulated, whereas the environmental information processing pathway in the ATP(R) group was significantly up-regulated (Figure 5A). The up-regulation of functional pathways in ATP(B) compared to that in ATP(R), TTG(R) and TTG(B) was mainly concentrated in genetic information processing pathways (Figure 5A). Moreover, the 41 functional pathways that differed between the groups were found at KEGG level 2 (Figure S4).

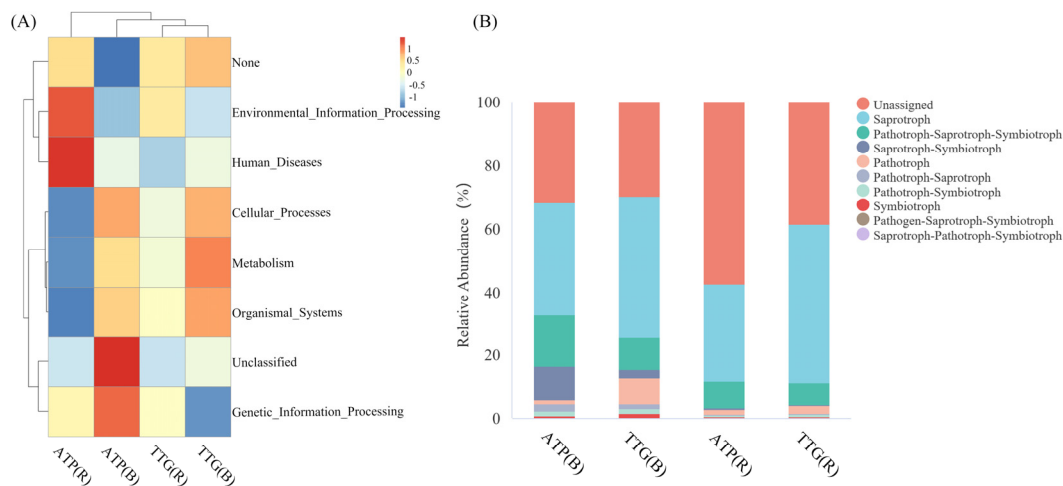


Figure 5. Heatmap of significantly different functional categories (at KEGG level 1) of bacterial communities predicted by PICRUSt (A) and relative abundance of corresponding fungal trophic modes predicted by the FUNGuild database (B). Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

The relative abundance of saprotroph in TTG(B) and TTG(R) was higher than that in ATP(B) and ATP(R), respectively, with the highest value of TTG(R) and the lowest value in ATP(R) (Figure 5B). However, the relative abundance of pathotroph-saprotroph-symbiotroph in ATP(B) and TTG(B) was higher than that in ATP(R) and TTG(R), respectively (Figure 5B).

3.5. Relationship between Soil Enzyme Activity, Soil Physicochemical Properties and Microbial Community Structure

In the rhizosphere soil of the two tea gardens, the bacterial community was not significantly correlated with soil enzyme activity and physicochemical properties (Figure 6A). But, the fungal community showed a strong positive correlation with soil pH, TP and DOC, whereas it was significantly negatively correlated with SWC, NO_3^- -N, NH_4^+ -N and βG (Figure 6A). However, in the bulk soil of the two tea gardens, the fungal community was found to have no significant correlation with soil enzyme activity and physicochemical properties (Figure 6B), whereas the bacterial community exhibited significant positive correlations with TC, TN, DOC, βG and ACP, representing the strongest correlation observed. Moreover, the bacterial community showed significant negative correlations with DON and the vector length (Figure 6B). PLS-PM analysis confirmed that soil properties directly influence fungal communities in the rhizosphere soil of tea gardens and bacterial communities in the bulk soil of tea gardens (Figure S5).

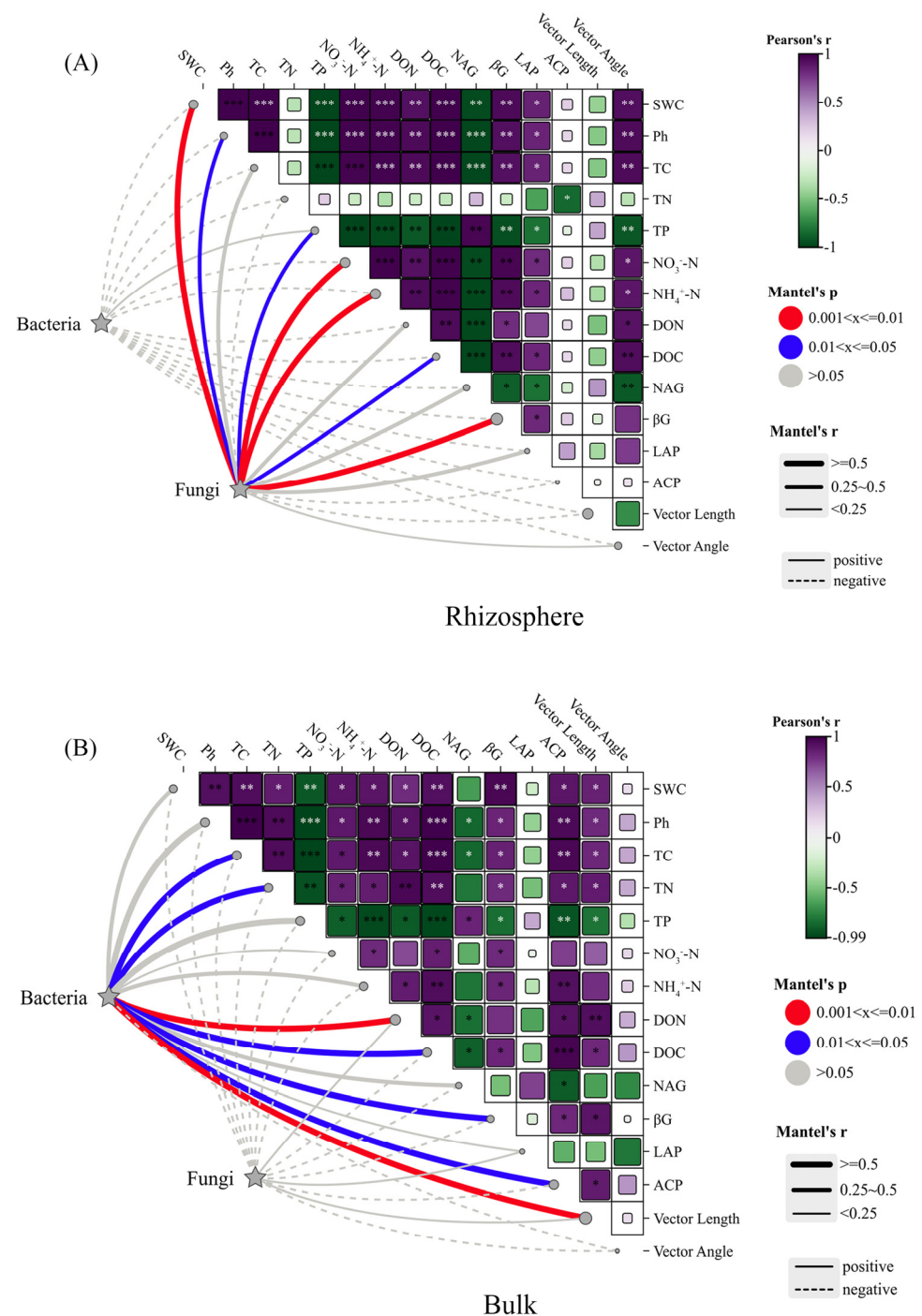


Figure 6. Mantel test between soil enzyme activity, soil physicochemical properties and microbial community structure (bacteria and fungi) in rhizosphere and bulk soil. The alpha diversity indices of bacteria (Shannon and Chao1), the three most abundant bacterial phyla (Proteobacteria, Acidobacteriota and Firmicutes) and the Bate diversity of bacteria (PCoA1 and PCoA2) represent bacterial communities. The alpha diversity indices of fungi (Shannon and Chao1), the three most abundant fungal phyla (Ascomycota, Basidiomycota and Mortierellomycota) and the Bate diversity of fungi (PCoA1 and PCoA2) represent fungal communities. Red lines indicate a significant negative correlation, whereas blue lines denote a significant positive correlation, and gray lines represent a nonsignificant correlation. Solid lines indicate positive Mantel's r values, whereas dashed lines indicate negative Mantel's r values. * indicates significance at the 0.05 level ($p < 0.05$), ** indicates significance at the 0.01 level ($p < 0.01$), *** indicates significance at the 0.001 level ($p < 0.001$).

3.6. Effects of Environmental Factors on Microbial Community at the Genera Level

Regarding the RDA analysis of soil bacterial communities, the first two axes explained 97.18% and 92.14% of the total variation in the bacterial communities of the rhizosphere and bulk soil, respectively, indicating that TN ($F = 7.6$, $p = 0.082$) appeared to be a major driving factor in the bacterial communities of the rhizosphere soil (Figure 7A), whereas $\text{NH}_4^+\text{-N}$ ($F = 6$, $p = 0.056$), SWC ($F = 4.2$, $p = 0.056$) and DON ($F = 5.6$, $p = 0.056$) were identified as the primary driving factors for the bacterial communities of the bulk soil (Figure 7B). Regarding the RDA analysis of soil fungal communities, the first two axes explained 98.71% and 85.23% of the total variation in the fungal communities of the rhizosphere and bulk soil, respectively, indicating that pH ($F = 27.3$, $p = 0.016$) and SWC ($F = 7$, $p = 0.096$) appeared to be major driving factor in the fungal communities of the rhizosphere soil (Figure 7C), and pH ($F = 10.7$, $p = 0.046$) appeared to be major driving factor in the fungal communities of the bulk soil (Figure 7D).

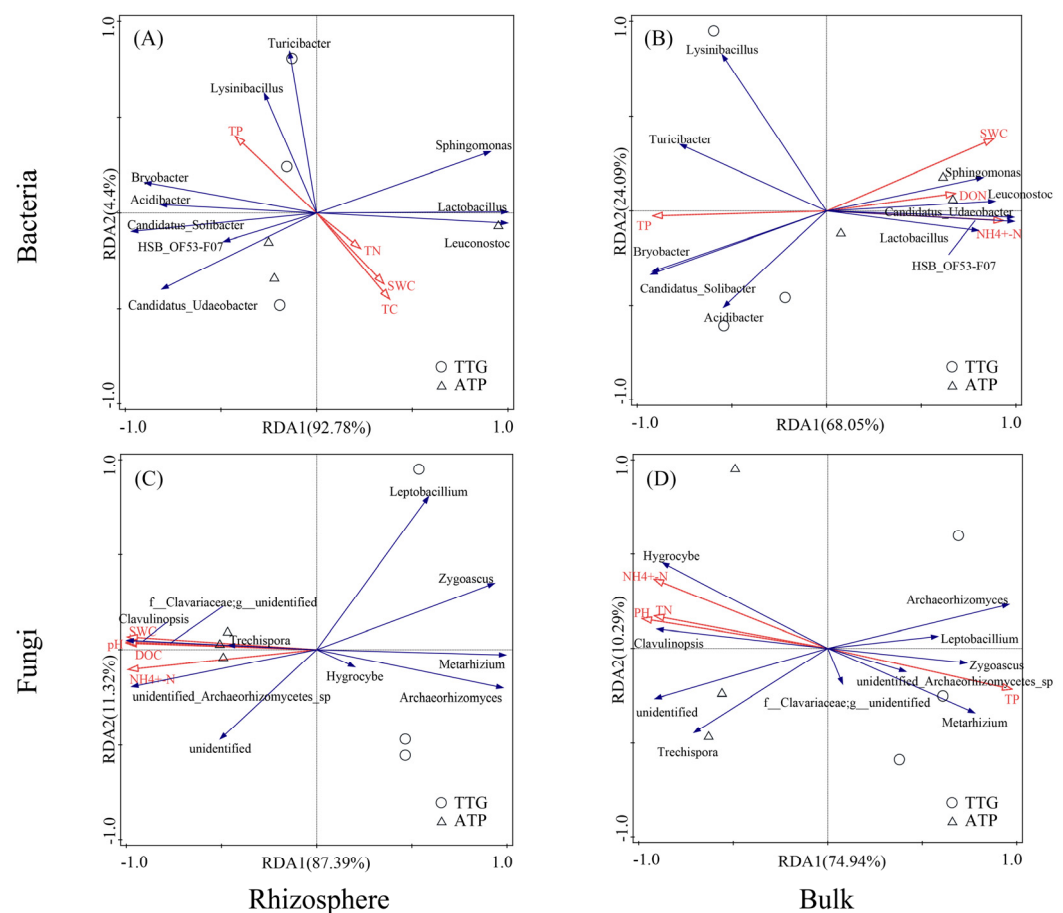


Figure 7. Redundancy analysis (RDA) illustrating the relationship between soil physicochemical properties and the bacterial (A,B) and fungal (C,D) communities of rhizosphere and bulk soil at the genera level. Sample abbreviations are as follows: TTG: tableland tea garden; ATP: ancient tea plantation.

4. Discussion

4.1. Changes in Soil Physicochemical Properties and Enzyme Activity among Different Tea Gardens

The rhizosphere is recognized as the zone of the most intense root activity and metabolism in the soil, serving as a crucial portal for nutrient cycling, where complex biological and ecological processes occur [27]. In this study, the physicochemical properties of rhizosphere soil in the ancient tea plantation were found to be higher than those in bulk soil, exhibiting a pronounced positive rhizospheric effect. However, in the tableland tea garden, the physicochemical properties of the soil only showed higher levels of soil SWC,

TN and DOC in the rhizosphere compared to the bulk soil (Table 1). This may be attributed to excessive anthropogenic disturbances to the tableland tea garden in pursuit of higher economic benefits, such as the overuse of chemical fertilizers and pesticides, which resulted in soil compaction, a reduced surface litter and significant disruption to the soil microecology. In contrast, the ancient tea plantation was subjected to minimal human influence, allowing its habitats to be conserved and contributing to a more pronounced rhizosphere effect. In the ancient tea plantation, a richer layer of surface litter was observed compared to that in the tableland tea garden. The higher elevation of the ancient tea plantation relative to the tableland tea garden resulted in lower temperatures (Table S1), which slowed down the decomposition rate of litter [28], thereby leading to greater accumulation of carbon. Kumar et al. (2019) [29] showed that elevation is positively correlated with soil pH and negatively correlated with TP, which is consistent with our study. Excessive fertilization and rainfall leaching in the tableland tea garden may have resulted in severe soil acidification and an elevated TP content, leading to progressive deterioration of soil quality that was detrimental to the growth of tea plants. However, the soil pH in the ancient tea plantation was found to attain optimal levels (4.5–5.5), which promoted their growth [30] and the accumulation of soil nutrient. Tea plants are an economically important crop primarily harvested for their leaves, preferring to absorb $\text{NH}_4^+\text{-N}$ rather than $\text{NO}_3^-\text{-N}$ from the soil [31]. However, the application of nitrogen fertilizers has been shown to enhance the nitrification efficiency of the conversion of $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ in soil [32]. In addition to the long-term application of chemical fertilizers in tableland tea gardens, differences in root exudates, leaf litter and microbial communities between tableland tea gardens and ancient tea plantations may also influence soil nitrification activity and rates. These factors may primarily account for the observed differences in the soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents between the two types of tea plantations.

Soil enzyme activities involved in the cycling of carbon (C), nitrogen (N) and phosphorus (P), as well as their stoichiometric ratios, are used to characterize the energy (C) and nutrient (N, P) limitations of soil microorganisms during growth and metabolism [20]. The rhizosphere is actually a dynamic system of interacting processes. In this study, the soil enzyme activities in the rhizosphere were found to be higher than those in bulk soils, indicating that plant rhizospheric microbial activity is more frequent, resulting in the release of more soil extracellular enzymes. This enhances the cycling of nutrients such as carbon, nitrogen and phosphorus while providing abundant nutrient support for both the microorganisms and tea plants. Wang et al. (2018) [33] found that soil enzyme activities related to carbon and nitrogen cycling increase with the age of tea plants, a phenomenon that is associated with the quality and quantity of tea plant litter. In our study, the ancient tea plantation experienced fewer anthropogenic disturbances compared to the tableland tea garden, and the abundant leaf litter on the ground enhanced the availability of soil organic matter [34], thereby influencing soil enzyme activity. Moreover, the abundant surface litter in the ancient tea plantation helped reduce the surface temperature and decrease soil compaction, as well as increase soil water retention capacity, which was beneficial for enhancing soil enzyme activity. Notably, enzyme activities related to nitrogen cycling (NAG) were significantly lower in the ancient tea garden compared to the tableland tea gardens in both rhizosphere and bulk soils, likely due to the long-term application of nitrogen fertilizers in the tableland tea garden [32]. Additionally, both of the tea gardens were experiencing varying degrees of carbon and nitrogen nutrient limitations.

4.2. Microbial Diversity and Composition in the Rhizosphere and Bulk Soil of Different Tea Gardens

In this study, the alpha diversity of the bacterial community was more responsive than that of the fungal community in both the tableland tea garden and the ancient tea plantation (Figure 2), in accordance with Delgado-Baquerizo et al. (2017) [35]. Moreover, the bacterial alpha diversity of the rhizosphere and bulk soil in the tableland tea garden was higher than that in the ancient tea plantation, suggesting that ancient tea trees create a relatively nutrient-rich ecological niche that imposes selective pressure on microorganisms, thereby

reducing their diversity [36]. Ancient tea plantations, as undisturbed forest stands with low-intensity production methods, are in a state of equilibrium, accompanied by accumulation on the forest floor and a reduction in competition [22]. Concurrently, the tableland tea garden was subject to more anthropogenic disturbances, particularly management practices such as fertilization, which may account for the higher bacterial and fungal alpha diversities compared to those of the ancient tea plantation [37,38]. In addition, the fungal alpha diversity of bulk soil in the ancient tea garden was significantly higher than that in its rhizosphere soil, which may be explained by a mechanism in which rhizosphere microbiomes are derived from bulk microbiomes under the selection effect of host plants [39]. Consistent diversity of the microbial communities was observed in the rhizosphere and bulk soil of the tableland tea garden, which was attributed to the severe acidification of the soil in the tea plantation [22]. Previous studies have shown that microbial community structures in rhizosphere soil are influenced by variations in aboveground plants [40]. This suggests that pruning in the tableland tea garden could induce alterations in microbial communities in the rhizosphere soil, whereas communities in the ancient tea garden were relatively stable, exerting minimal impact on the subterranean microbial communities.

The bacterial communities were largely dominated by *Proteobacteria* and *Acidobacteriota* (Figure 2A), whereas the fungal communities were largely dominated by Ascomycota and Basidiomycota in the rhizosphere and bulk soil of the two tea plantations (Figure 2B). In previous studies on tea plantation soils, these results have been confirmed to be commonly prevalent [11,41,42]. Furthermore, the relative abundance of *Firmicutes* was found to be highest in the rhizosphere soil of the ancient tea plantation, consistent with findings reported by Chen et al. (2021) [43], who reported that the relative abundance of *Firmicutes* was observed to increase with the age of the tea plantation. Under varying soil nutrient conditions in rhizosphere and bulk soils, microbial taxa exhibit distinct ecological strategies and manifest as different abundant groups [22]. *Acidobacteria* and *Proteobacteria* are recognized as important contributors to the soil carbon cycle. *Acidobacteria* prefer acidic conditions and degrade cellulose and lignin in plant debris within resource-poor soils, whereas *Proteobacteria* utilize labile carbon sources in nutrient-rich environments [42,44]. In this study, the abundance of *Proteobacteria* was higher in the rhizosphere soil of both the tableland tea garden and the ancient tea plantation compared to their respective bulk soils, with the highest abundance observed in the rhizosphere of the ancient tea plantation, indicating that the nutrient-rich environment of the ancient tea plantation's rhizosphere enhances the growth of *Proteobacteria*, being particularly conducive to the growth of microbes. *Acidobacteria*, typically oligotrophic organisms, were found to be more abundant in the acidic bulk soils of the tableland tea garden (Figure 3A).

Furthermore, the dominant phyla in the fungal communities were Ascomycota and Basidiomycota, and their changes drove differences in community structures among tea garden soil (Figure 3B). Plant residues are decomposed by Ascomycota, releasing carbon, nitrogen and other nutrients that provide nourishment for soil microorganisms and plants, positioning them as the principal fungal decomposers in the soil; Basidiomycota decompose complex compounds within plant residues, such as lignin, which release nutrients for utilization by other organisms, thereby facilitating the carbon and organic matter cycles in the soil [41,45,46]. Our results show that Ascomycota occupied a substantial proportion of the soil microbial communities in both tea gardens; the abundance of Ascomycota in the rhizosphere soils of the two tea gardens was observed to be greater than that in their respective bulk soils. Moreover, a higher abundance was found in the tableland tea garden soil as compared to that of the ancient tea plantation. This indicates that a greater quantity of labile carbon sources was present in the rhizosphere soil of the tea plantation, which favored the growth of and nutrient release by Ascomycota, and the nutrient cycling rate in the soils of tableland tea garden appeared to be more rapid. However, the abundance of Basidiomycete in the bulk soil of the tea gardens exceeded that found within their rhizosphere soil. Concurrently, a significantly higher abundance of Basidiomycete was found in both the rhizosphere and bulk soil of the ancient tea plantation compared to that

of the tableland tea garden. These findings indicate that the ancient tea plantations had a more extensive coverage of surface litter, contributing to a more favorable soil ecology than that found in the tableland tea garden.

4.3. Microbial Communities and Functional Groups in Rhizosphere and Bulk Soil of Different Tea Gardens

Microbial functional groups are instrumental to enhancing our understanding of the ecological implications of microbial responses to soils from different tea plantations, as well as being crucial for the balance of soil nutrients and the growth and development of tea plants [47,48]. Based on the KEGG database, functional predictions of high accuracy were conducted by PICRUSt utilizing 16S bacterial sequencing data. The results showed that the abundance of various functions in both the rhizosphere and bulk soil of the two tea plantations differed to varying extents, but the proportion of metabolic functions was highest among them. Bhattacharyya et al. (2022) [49] confirmed that, compared to geographic location and soil type, the rhizosphere of tea plantations has the greatest influence on metabolic pathways. Interestingly, the metabolic pathway of the bacterial community in the rhizosphere soil of the ancient tea plantation was significantly down-regulated compared to other soils, whereas the environmental information processing pathway was significantly up-regulated (Figure 5A). This may be mainly attributed to land use changes [47], where the soil of the ancient tea plantation was significantly influenced by the root systems of tea trees and was subjected to minimal human disturbance, leading to a more stable soil microbial community. In addition, Amino_Acid_Metabolism, Energy_Metabolism, Transport_and_Catabolism, Carbohydrate_Metabolism and Glycan_Biosynthesis_and_Metabolism were significantly down-regulated (at KEGG level 2) in the rhizosphere soil of the ancient tea plantation compared to other soils (Figure S3). A previous study also suggested that microorganisms have different metabolic mechanisms to adapt to the ecological impact in the rhizosphere soil of Pu-erh tea [50].

Soil fungal communities are an important component of nutrient cycling within soil, and nutrient types in tea plantation soils are predicted based on the FUNGuild database. In this study, the abundant functional genes of fungi in tea plantations were predominantly concentrated in saprotrophs, pathotroph-saprotroph-symbiotrophs and saprotroph-symbiotrophs (Figure 5B). Saprotrophic fungi feed on decomposing dead organic matter and are able to convert complex organic molecules (such as cellulose, lignin and proteins) into simpler compounds [51]. Low-nutrient environments are conducive to stimulating the growth of saprotrophic fungi [52]. In this study, the relative abundance of saprotrophs in both the rhizosphere and bulk soil of the tableland tea garden was higher than that in the ancient tea plantation, with the greatest concentration found in the rhizosphere soil of the tableland tea garden. This is conducive to enhancing the decomposition of plant residues in the soils of the tableland tea garden, thereby accumulating more nutrients. Additionally, pathogenic fungi acquire nutrients by attacking host cells, leading to adverse effects on plant–fungus interactions and impeding plant growth [53]. This study reveals that the abundance of pathotroph-saprotroph-symbiotrophs was higher in bulk soil with relatively low nutrient levels in the two tea gardens but lower in the rhizosphere soil, suggesting a healthier state of the rhizospheric fungal community in tea gardens and a reduced risk of restricted tea plant growth, consistent with the findings by Du et al. (2022) [54].

4.4. Contrasting Drivers of Bacterial and Fungal Communities in the Rhizosphere and Bulk Soil of Tea Gardens

Previous studies have revealed that the selectivity of the soil environment is less for bacteria than fungi, resulting in an increased migration rate of bacterial communities [55]. Our findings demonstrate that the structure and composition of soil bacterial communities in the tea gardens were similar, but substantial differences were observed in the fungal communities (Figure 4), suggesting that soil bacteria are better adapted to homogenous environments and are less influenced by specific processes within tea gardens. The in-

fluence of ecological niches, such as rhizosphere and bulk soil, on bacterial and fungal communities in tea gardens differs, consistent with our second hypothesis. The Mantel test revealed that the bacterial communities showed no significant correlation with soil physicochemical properties and enzyme activities, but fungal communities were influenced by multiple factors in the rhizosphere soil of the tea gardens. This may have been associated with the management practices of the tea gardens, partially confounding the effects of the rhizosphere on the bacterial communities [56], whereas fungal communities were more sensitive to environmental changes. In addition, the role of arbuscular mycorrhizal fungi (AMF), such as the *Glomus* and *Acaulospora* genera present in tea garden soils, is significant [57]. They establish symbiotic relationships with tea tree roots, aiding in nutrient uptake, especially phosphorus, from the soil, which enhances tea tree growth and resilience to environmental challenges [10]. In the realm of soil science, the presence of AMF is acknowledged for its pivotal contribution to soil health and fertility, promoting sustainable agricultural practices within tea gardens. In this study, the relative deficiency of nutrients in the bulk soil of the tea gardens may have been one of the reasons why bacterial communities were found to be significantly positively correlated with soil TC, TN, DOC, β G and ACP. To explain the above observation, our PLS-PM analysis confirmed that the soil properties directly affected fungal communities in the rhizosphere soil of the tea gardens and bacterial communities in the bulk soil of the tea gardens (Figure S5), consistent with our third hypothesis. Thus, the redundancy analysis (RDA) further revealed that soil TN ($F = 7.6$, $p = 0.082$) exerted the greatest influence on the bacterial genera level in the rhizosphere soil of the tea gardens, whereas soil $\text{NH}_4^+\text{-N}$ ($F = 6$, $p = 0.056$), SWC ($F = 4.2$, $p = 0.056$) and DON ($F = 5.6$, $p = 0.056$) had the most significant impact on the bacterial genera level in the bulk soil of the tea gardens. It is noteworthy that soil pH was a key factor influencing the fungal genera level in both the rhizosphere and bulk soil in the tea gardens, consistent with previous research findings [58,59]. Nevertheless, the lack of a correlation between soil pH and bacterial communities in the rhizosphere and bulk may be attributed to the narrow range of soil pH values observed [3]. In addition, the preference of tea trees for acidic conditions, as well as Acidobacteria being the dominant bacterial phylum in the tea plantation soils, may serve as another reason. The aforementioned results do not align with our first hypothesis, indicating that microbial communities in rhizosphere and bulk soils are influenced by distinct factors, rather than a stronger interaction between the rhizosphere and microbes. Moreover, only three replicates were established in this study due to the constraints of topographic conditions; a broader and more fine-grained spatial extent during the sampling process is required to validate the findings of this research. The results may have been influenced by different microbial community analysis methods, which did not provide direct evidence in this study. Therefore, future studies should further focus on the roles and mechanisms of different bacterial and fungal communities in tea garden soils.

5. Conclusions

This study provides insight into the differences and driving factors in microbial communities between rhizosphere and bulk soil in different tea gardens. Based on our results, the rhizosphere effect of soil nutrients was observed to be more significant in the ancient tea plantation, whereas soil nutrients in the tableland tea garden were found to be comparatively deficient. Enzyme activities in the rhizosphere soil of both tea gardens were higher than those in the bulk soil, and both soils in the two tea gardens were subjected to a certain degree of C&N limitation. The differences in the microbial communities between the tableland tea garden and the ancient tea plantation are attributed to factors such as management practices, the size of tea plants, elevation and geographic location. In addition, soil pH and SWC were the key factors influencing the fungal communities in both the rhizosphere and bulk soil in the tea gardens, whereas bacterial communities were more significantly affected by soil TN, $\text{NH}_4^+\text{-N}$, SWC and DON. Taken together, this study recommends that interventions be implemented in tableland tea gardens, such as rational

deep tillage and the application of farmyard manure and compost, to improve soil quality, while advising proper pruning for ancient tea plantations. Our research provides the necessary foundational information for the preservation of ancient tea plantations, the ecological adaptability of ancient tea trees and the management of tableland tea gardens.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14071388/s1>: Table S1. Characteristics of tea gardens; Table S2. Soil enzyme activities in different tea gardens; Figure S1. Rarefaction curves of soil bacterial (A) and fungal (B) communities; Figure S2. Venn graph of soil bacterial (A) and fungal (B) communities; Figure S3. Linear discriminant analysis effect size (LEfSe) method showing the significantly distinct taxa of bacteria (A, B) and fungi (C, D) across TTG(R), TTG(B), ATP(R) and ATP(B); Figure S4. Heatmap of the significantly different functional categories (at KEGG level 2) of bacterial communities predicted by PICRUSt; Figure S5. Path analysis diagrams between soil enzyme activity, soil physicochemical properties and microbial community structure (bacteria and fungi) in rhizosphere and bulk soil based on the partial least squares path model (PLS-PM).

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References

1. Qi, D.H.; Guo, H.J.; Sheng, C.Y. Assessment of plant species diversity of ancient tea garden communities in Yunnan, Southwest of China. *Agroforest. Syst.* **2013**, *87*, 465–474. [\[CrossRef\]](#)
2. Wang, F.; Cheng, X.; Cheng, S.; Li, W.; Huang, X. Genetic diversity of the wild ancient tea tree (*Camellia taliensis*) populations at different altitudes in Qianjiazhai. *PLoS ONE* **2023**, *18*, e0283189. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Zi, H.; Jiang, Y.; Cheng, X.; Li, W.; Huang, X. Change of rhizospheric bacterial community of the ancient wild tea along elevational gradients in Ailao mountain, China. *Sci. Rep.* **2020**, *10*, 9203. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Li, W.; Zhang, Q.; Fan, Y.; Cheng, Z.; Lu, X.; Luo, B.; Long, C. Traditional management of ancient Pu'er teagardens in Jingmai Mountains in Yunnan of China, a designated Globally Important Agricultural Heritage Systems site. *J. Ethnobiol. Ethnomed.* **2023**, *19*, 26. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Ludwig, M.; Achtenhagen, J.; Miltner, A.; Eckhardt, K.U.; Leinweber, P.; Emmerling, C.; Thiele-Bruhn, S. Microbial contribution to SOM quantity and quality in density fractions of temperate arable soils. *Soil Biol. Biochem.* **2015**, *81*, 311–322. [\[CrossRef\]](#)
6. Waldrop, M.P.; Holloway, J.M.; Smith, D.B.; Goldhaber, M.B.; Drenovsky, R.E.; Scow, K.M.; Grace, J.B. The interacting roles of climate, soils, and plant production on soil microbial communities at a continental scale. *Ecology* **2017**, *98*, 1957–1967. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Wang, X.; Tang, C.; Severi, J.; Butterly, C.R.; Baldock, J.A. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. *New Phytol.* **2016**, *211*, 864–873. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Li, W.; Zheng, Z.; Li, T.; Zhang, X.; Wang, Y.; Yu, H.; Liu, T. Effect of tea plantation age on the distribution of soil organic carbon fractions within water-stable aggregates in the hilly region of Western Sichuan, China. *Catena* **2015**, *133*, 198–205. [\[CrossRef\]](#)
9. Sun, C.; Wang, D.; Shen, X.; Li, C.; Liu, J.; Lan, T.; Zhang, Y. Effects of biochar, compost and straw input on root exudation of maize (*Zea mays* L.): From function to morphology. *Agric. Ecosyst. Environ.* **2020**, *297*, 106952. [\[CrossRef\]](#)
10. Bag, S.; Mondal, A.; Banik, A. Exploring tea (*Camellia sinensis*) microbiome: Insights into the functional characteristics and their impact on tea growth promotion. *Microbiol. Res.* **2022**, *254*, 126890. [\[CrossRef\]](#)
11. Yang, G.; Zhou, D.; Wan, R.; Wang, C.; Xie, J.; Ma, C.; Li, Y. HPLC and high-throughput sequencing revealed higher tea-leaves quality, soil fertility and microbial community diversity in ancient tea plantations: Compared with modern tea plantations. *BMC Plant Biol.* **2022**, *22*, 239. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Gao, Z.; Karlsson, I.; Geisen, S.; Kowalchuk, G.; Jousset, A. Protists: Puppet masters of the rhizosphere microbiome. *Trends Plant Sci.* **2019**, *24*, 165–176. [\[CrossRef\]](#) [\[PubMed\]](#)

13. Ding, L.J.; Cui, H.L.; Nie, S.A.; Long, X.E.; Duan, G.L.; Zhu, Y.G. Microbiomes inhabiting rice roots and rhizosphere. *FEMS Microbiol. Ecol.* **2019**, *95*, fiz040. [[CrossRef](#)] [[PubMed](#)]
14. Tian, T.; Reverdy, A.; She, Q.; Sun, B.; Chai, Y. The role of rhizodeposits in shaping rhizomicrobiome. *Environ. Microbiol. Rep.* **2020**, *12*, 160–172. [[CrossRef](#)] [[PubMed](#)]
15. Xie, H.; Chen, Z.; Feng, X.; Wang, M.; Luo, Y.; Wang, Y.; Xu, P. L-theanine exuded from *Camellia sinensis* roots regulates element cycling in soil by shaping the rhizosphere microbiome assembly. *Sci. Total Environ.* **2022**, *837*, 155801. [[CrossRef](#)] [[PubMed](#)]
16. Chialva, M.; Lanfranco, L.; Bonfante, P. The plant microbiota: Composition, functions, and engineering. *Curr. Opin. Biotechnol.* **2022**, *73*, 135–142. [[CrossRef](#)] [[PubMed](#)]
17. Wang, H.B.; Zhang, Q.X.; Chen, X.T.; Wang, Y.H.; Lin, L.W.; Ye, J.H.; He, H.B. Analysis of microbial diversity of tea tree (*Camellia sinensis* (L.) O. Ktze.) sick rhizospheric soil using soil metaproteomic technology. *Allelopath. J.* **2020**, *51*, 147–156. [[CrossRef](#)]
18. Li, J.Y.; Chen, P.; Li, Z.G.; Li, L.Y.; Zhang, R.Q.; Hu, W.; Liu, Y. Soil aggregate-associated organic carbon mineralization and its driving factors in rhizosphere soil. *Soil Biol. Biochem.* **2023**, *186*, 109182. [[CrossRef](#)]
19. Kotroczó, Z.; Veres, Z.; Fekete, I.; Krakomperger, Z.; Tóth, J.A.; Lajtha, K.; Tóthmérész, B. Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biol. Biochem.* **2014**, *70*, 237–243. [[CrossRef](#)]
20. Moorhead, D.L.; Sinsabaugh, R.L.; Hill, B.H.; Weintraub, M.N. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Biol. Biochem.* **2016**, *93*, 1–7. [[CrossRef](#)]
21. Qin, M.; Zhang, Q.; Pan, J.; Jiang, S.; Liu, Y.; Bahadur, A.; Feng, H. Effect of arbuscular mycorrhizal fungi on soil enzyme activity is coupled with increased plant biomass. *Eur. J. Soil Sci.* **2020**, *71*, 84–92. [[CrossRef](#)]
22. Wang, Y.; Jiao, P.; Guo, W.; Du, D.; Hu, Y.; Tan, X.; Liu, X. Changes in bulk and rhizosphere soil microbial diversity and composition along an age gradient of Chinese fir (*Cunninghamia lanceolata*) plantations in subtropical China. *Front. Microbiol.* **2022**, *12*, 777862. [[CrossRef](#)]
23. Li, J.; Yuan, X.; Ge, L.; Li, Q.; Li, Z.; Wang, L.; Liu, Y. Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification. *Agric. Ecosyst. Environ.* **2020**, *304*, 107126. [[CrossRef](#)]
24. Sinsabaugh, R.L.; Hill, B.H.; Follstad Shah, J.J. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* **2009**, *462*, 795–798. [[CrossRef](#)] [[PubMed](#)]
25. Xie, Y.; Ouyang, Y.; Han, S.; Se, J.; Tang, S.; Yang, Y.; Wu, L. Crop rotation stage has a greater effect than fertilisation on soil microbiome assembly and enzymatic stoichiometry. *Sci. Total Environ.* **2022**, *815*, 152956. [[CrossRef](#)]
26. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, 1–18. [[CrossRef](#)]
27. Bais, H.P.; Weir, T.L.; Perry, L.G.; Gilroy, S.; Vivanco, J.M. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 233–266. [[CrossRef](#)]
28. Yükses, T.; Yükses, F. Effects of altitude, aspect, and soil depth on carbon stocks and properties of soils in a tea plantation in the humid Black Sea region. *Land Degrad. Dev.* **2021**, *32*, 4267–4276. [[CrossRef](#)]
29. Kumar, S.; Suyal, D.C.; Yadav, A.; Shouche, Y.; Goel, R. Microbial diversity and soil physiochemical characteristic of higher altitude. *PLoS ONE* **2019**, *14*, e0213844. [[CrossRef](#)] [[PubMed](#)]
30. Ye, J.; Wang, Y.; Wang, Y.; Hong, L.; Jia, X.; Kang, J.; Wang, H. Improvement of soil acidification in tea plantations by long-term use of organic fertilizers and its effect on tea yield and quality. *Front. Plant Sci.* **2022**, *13*, 1055900. [[CrossRef](#)]
31. Yang, Y. *China Tea Cultivation*; Shanghai Scientific and Technical Publishers: Shanghai, China, 2005.
32. Xue, D.; Yao, H.; Huang, C. Microbial biomass, N mineralization and nitrification, enzyme activities, and microbial community diversity in tea orchard soils. *Plant Soil.* **2006**, *288*, 319–331. [[CrossRef](#)]
33. Wang, S.; Li, T.; Zheng, Z. Effects of tea plantation age on soil aggregate-associated C-and N-cycling enzyme activities in the hilly areas of Western Sichuan, China. *Catena* **2018**, *171*, 145–153. [[CrossRef](#)]
34. Cotrufo, M.F.; Wallenstein, M.D.; Boot, C.M.; Deneff, K.; Paul, E. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Glob. Change Biol.* **2013**, *19*, 988–995. [[CrossRef](#)]
35. Delgado-Baquerizo, M.; Reich, P.B.; Khachane, A.N.; Campbell, C.D.; Thomas, N.; Freitag, T.E.; Singh, B.K. It is elemental: Soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* **2017**, *19*, 1176–1188. [[CrossRef](#)] [[PubMed](#)]
36. Li, H.; Xu, Z.; Yan, Q.; Yang, S.; Van Nostrand, J.D.; Wang, Z.; Deng, Y. Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland. *Plant Soil* **2018**, *423*, 465–480. [[CrossRef](#)]
37. Li, J.; Li, S.; Huang, X.; Tang, R.; Zhang, R.; Li, C.; Su, J. Plant diversity and soil properties regulate the microbial community of monsoon evergreen broad-leaved forest under different intensities of woodland use. *Sci. Total Environ.* **2022**, *821*, 153565. [[CrossRef](#)]
38. Zhang, Z.; Ge, S.; Fan, L.C.; Guo, S.; Hu, Q.; Ahammed, G.J.; Li, X. Diversity in rhizospheric microbial communities in tea varieties at different locations and tapping potential beneficial microorganisms. *Front. Microbiol.* **2022**, *13*, 1027444. [[CrossRef](#)]
39. Yang, Y.; Hao, Z.; Wei, L.; Jiamei, S.; Zhao, M.; Han, G.; Pan, Q. Effects of grazing intensity on richness and composition of rhizosphere and non-rhizosphere microbial communities in a semiarid grassland. *Authorea Prepr.* **2022**. [[CrossRef](#)]
40. Nan, J.; Chao, L.; Ma, X.; Xu, D.; Mo, L.; Zhang, X.; Bao, Y. Microbial diversity in the rhizosphere soils of three *Stipa* species from the eastern Inner Mongolian grasslands. *Glob. Ecol. Conserv.* **2020**, *22*, e00992. [[CrossRef](#)]

41. Gu, S.; Hu, Q.; Cheng, Y.; Bai, L.; Liu, Z.; Xiao, W.; Tan, L. Application of organic fertilizer improves microbial community diversity and alters microbial network structure in tea (*Camellia sinensis*) plantation soils. *Soil Tillage Res.* **2019**, *195*, 104356. [\[CrossRef\]](#)
42. Bai, Y.C.; Li, B.X.; Xu, C.Y.; Raza, M.; Wang, Q.; Wang, Q.Z.; Xu, Y.J. Intercropping walnut and tea: Effects on soil nutrients, enzyme activity, and microbial communities. *Front. Microbiol.* **2022**, *13*, 852342. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Chen, P.; Liu, Y.; Mo, C.; Jiang, Z.; Yang, J.; Lin, J. Microbial mechanism of biochar addition on nitrogen leaching and retention in tea soils from different plantation ages. *Sci. Total Environ.* **2021**, *757*, 143817. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Zhang, S.; Wang, Y.; Sun, L.; Qiu, C.; Ding, Y.; Gu, H.; Ding, Z. Organic mulching positively regulates the soil microbial communities and ecosystem functions in tea plantation. *BMC Microbiol.* **2020**, *20*, 103. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Li, Y.; Li, Z.; Arafat, Y.; Lin, W. Studies on fungal communities and functional guilds shift in tea continuous cropping soils by high-throughput sequencing. *Ann. Microbiol.* **2020**, *70*, 7. [\[CrossRef\]](#)
46. Kui, L.; Xiang, G.; Wang, Y.; Wang, Z.; Li, G.; Li, D.; Dong, Y. Large-scale characterization of the soil microbiome in ancient tea plantations using high-throughput 16S rRNA and internal transcribed spacer amplicon sequencing. *Front. Microbiol.* **2021**, *12*, 745225. [\[CrossRef\]](#) [\[PubMed\]](#)
47. He, H.; Miao, Y.; Gan, Y.; Wei, S.; Tan, S.; Rask, K.A.; Ekelund, F. Soil bacterial community response to long-term land use conversion in Yellow River Delta. *Appl. Soil Ecol.* **2020**, *156*, 103709. [\[CrossRef\]](#)
48. Yu, C.; Cao, J.; Du, W.; Zhu, Z.; Xu, M. Changes in the population and functional profile of bacteria and fungi in the rhizosphere of *Suaeda salsa* is driven by invasion of *Spartina alterniflora*. *Ecol. Indic.* **2022**, *144*, 109516. [\[CrossRef\]](#)
49. Bhattacharyya, C.; Imchen, M.; Mukherjee, T.; Halder, S.; Mondal, S.; Mukherji, S.; Ghosh, A. Rhizosphere impacts bacterial community structure in the tea (*Camellia sinensis* (L.) O. Kuntze.) estates of Darjeeling, India. *Environ. Microbiol.* **2022**, *24*, 2716–2731. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Chen, Y.; Li, Q.; Wu, W.; Liu, X.; Cheng, J.; Deng, X.; Wang, B. Effects of lightning on rhizosphere soil properties, bacterial communities, and active components of *Camellia sinensis* var. *assamica*. *Front. Microbiol.* **2022**, *13*, 911226. [\[CrossRef\]](#)
51. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [\[CrossRef\]](#)
52. Fan, D.; Zhao, Z.; Wang, Y.; Ma, J.; Wang, X. Crop-type-driven changes in polyphenols regulate soil nutrient availability and soil microbiota. *Environ. Microbiol.* **2022**, *13*, 964039. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Guo, X.P.; Yang, Y.; Niu, Z.S.; Lu, D.P.; Zhu, C.H.; Feng, J.N.; Hou, L. Characteristics of microbial community indicate anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area, China. *Sci. Total Environ.* **2019**, *648*, 306–314. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Du, L.; Zheng, Z.; Li, T.; Wang, Y.; Huang, H.; Yu, H.; Zhang, X. Variations of fungal communities within the soils of different tea varieties (*Camellia sinensis* L.) following long-term plantation. *Plant Soil* **2022**, *477*, 665–677. [\[CrossRef\]](#)
55. Li, J.; Yang, L.; Mao, S.; Fan, M.; Shangguan, Z. Assembly and enrichment of rhizosphere and bulk soil microbiomes in *Robinia pseudoacacia* plantations during long-term vegetation restoration. *Appl. Soil Ecol.* **2023**, *187*, 104835. [\[CrossRef\]](#)
56. Singh, B.K.; Munro, S.; Potts, J.M.; Millard, P. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Appl. Soil Ecol.* **2007**, *36*, 147–155. [\[CrossRef\]](#)
57. Chen, Y.; Fu, W.; Xiao, H.; Zhai, Y.; Luo, Y.; Wang, Y.; Huang, J. A Review on Rhizosphere Microbiota of Tea Plant (*Camellia sinensis* L.): Recent Insights and Future Perspectives. *J. Agric. Food Chem.* **2023**, *71*, 19165–19188. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Yang, W.; Li, C.; Wang, S.; Zhou, B.; Mao, Y.; Rensing, C.; Xing, S. Influence of biochar and biochar-based fertilizer on yield, quality of tea and microbial community in an acid tea orchard soil. *Appl. Soil Ecol.* **2021**, *166*, 104005. [\[CrossRef\]](#)
59. Ji, L.; Ni, K.; Wu, Z.; Zhang, J.; Yi, X.; Yang, X.; Ruan, J. Effect of organic substitution rates on soil quality and fungal community composition in a tea plantation with long-term fertilization. *Biol. Fertil. Soils* **2020**, *56*, 633–646. [\[CrossRef\]](#)

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