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Renewal and Iteration Mechanisms of Aged Tea Trees: Insights from Tea Garden Soil Microbial Communities

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Abstract: This study focuses on the renewal and iteration mechanisms of aged tea trees in interactions with their soil microbial communities, aiming to elucidate the impact of the planting age of tea trees on the structure and function of soil microbial communities and how these impacts are linked to the formation of tea quality. By conducting a comparative analysis of the cultivation soil from tea trees with varying planting ages ranging from 30 to 200 years, we employed microbial diversity sequencing, a soil physicochemical property analysis, and tea leaf chemical component detection. We combined these methods with redundancy analysis (RDA) and linear discriminant analysis effect size (LEfSe) to reveal significant correlations between the planting age of tea trees and the soil's microbial diversity and function. The results indicate that as the planting age of tea trees increases, there are significant changes in the soil's pH and nutrient content. Concurrently, the components of the tea leaves also change. Most notably, around the 120 years mark of the tea tree planting age, the diversity of the soil microbial community reaches a turning point. Key microbial community analyses revealed shifts in the dominant microbial populations within the soil across the various tea tree planting ages, exemplified by taxa such as Hygrocybe Mycena, Humicola, Bradyrhizobium, and Candidatus Solibacter. These alterations in microbial communities are closely associated with soil nutrient dynamics and the developmental stages of tea trees. These findings not only provide scientific guidance for tea garden management, tea tree cultivation, and tea production but also offer new insights into the impact of tea tree–soil–microbe interactions on tea quality, which is significantly important for enhancing tea quality.

Keywords: microbial diversity; soil microbial communities; soil nutrient dynamics; tea quality formation; tea tree planting age

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Citation: Wang, H.; Wu, T.; Yuan, W.; Chen, L.; Li, H.; Deng, X.; Wang, C.; Liu, W.; Huang, W.; Wang, B. Renewal and Iteration Mechanisms of Aged Tea Trees: Insights from Tea Garden Soil Microbial Communities. *Agronomy* **2024**, *14*, 2955. https://doi.org/10.3390/agronomy14122955

Academic Editor: Robert P. Larkin

Received: 14 October 2024
Revised: 7 December 2024
Accepted: 10 December 2024
Published: 12 December 2024

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1. Introduction

Yunnan ancient tea trees hold a significant position in the international tea market due to their unique flavor and profound cultural heritage [1]. These ancient tea trees are not only living fossils of tea culture heritage but are also key resources for exploring the growth, development, and quality formation mechanisms of tea trees [2]. The age of Yunnan's ancient tea trees generally exceeds a century, with some even reaching a millennium. Throughout their long process of natural selection and artificial cultivation, they have formed a unique growing environment and a symbiotic system with microorganisms [3]. The in-depth exploration of how these ancient tea trees achieve renewal and iteration as they age has great scientific value and practical significance for understanding the growth mechanisms, the quality formation, and the mechanisms of interaction between tea trees and the environment.

Plant renewal and iteration are key links in its life cycle, involving a series of complex processes such as growth, reproduction, senescence, and regeneration [4]. Plants maintain long-term vitality through strategies such as cambium activity and root regeneration [5]. At the molecular level, plant hormones such as auxins, cytokinins, and ethylene play a central role in regulating plant growth, differentiation, and senescence [6,7]. Meanwhile, DNA methylation and microRNA, as well as other epigenetic modifications, are closely related to a plant's aging and regenerative capacity [8–10]. In recent years, researchers have also begun to focus on how plants adjust metabolic pathways and antioxidant defense systems to adapt to environmental changes, thereby maintaining vitality. As plants age, they must adapt to environmental changes to maintain vitality and productivity [11,12]. In this process, the interaction between the plant's roots and the soil microorganisms is particularly important. Soil microbial communities not only participate in nutrient cycling and promote plant nutrient absorption but also participate in the synthesis of plant hormones and the improvement of the soil structure, thus affecting plant health and growth [13,14]. Over time, plants provide a rich carbon source for soil microorganisms through root exudates, leaf litter, and root shedding, further shaping the structure and function of rhizosphere microbial communities, while also changing plant secondary metabolites [15,16].

Secondary metabolites play a crucial role in the renewal and iteration process of plants, especially in dealing with environmental stress and pathogen attacks [17,18]. For tea trees, secondary metabolites such as tea polyphenols, catechins, and theanine not only determine the flavor and quality of tea leaves but are also closely related to the adaptability and growth condition of tea trees [19]. Studies have shown that the nitrogen, phosphorus, and potassium content in tea tree leaves are important indicators reflecting the nutritional status and growth vitality of tea trees. The nitrogen content in tea tree leaves is closely related to the growth rate and photosynthesis intensity of tea trees, while phosphorus and potassium are related to the stress resistance and root development of tea trees. For example, a study on Yunnan ancient tea trees found that as the tree age increases, the nitrogen content in the tea leaves decreases, while the potassium content increases, which may be related to the adjustment of the tea trees' nutrient absorption and distribution strategies [20]. In addition, the nitrogen, phosphorus, and potassium content in tea trees is also related to the composition of root exudates, which can affect the composition and activity of rhizosphere microorganisms, thereby affecting the nutrient absorption and growth of tea trees [21].

Soil microbial communities are closely related to soil nutrient cycling and have a significant impacts on the renewal and iteration and growth of tea trees. Soil microorganisms decompose organic matter to release nutrients and participate in the transformation and cycling processes of nitrogen, phosphorus, potassium, and other elements in the soil, thus affecting the nutritional status of tea trees [22]. In the field of tea garden soil microbiology, existing research has demonstrated that rhizosphere microbial communities play a significant role in the health and growth of tea plants. Typical rhizosphere bacteria include Pseudomonas, Rhizobium, and Bacillus, while common fungi comprise Arbuscular Mycorrhizal Fungi and Ectomycorrhizal Fungi. These microorganisms are involved in soil nutrient cycling, the synthesis of plant hormones, and the improvement of soil structure, thereby influencing the growth of tea plants and the quality of tea leaves [23,24].

This study aims to investigate how the structure and function of soil microbial communities in Yunnan ancient tea gardens change with the increase of the tea tree planting age and how these changes are associated with the soil's nutrient status and the renewal and iteration of the tea trees. By integrating the analysis of the soil microbial composition, nutrient content, and tea tree growth status, this study reveals how soil microbes and nutrients jointly influence the renewal and iteration process of tea trees. This study provides a new perspective for future research on the interaction between tea tree growth and soil microorganisms, enhancing our understanding of the mechanisms of interaction between tea trees, soil, and microorganisms.

2. Materials and Methods

2.1. Plant and Soil Materials

This study was conducted in a tea garden located at the research base of Yunnan Agricultural University in Yunnan Province, China. The plantations involved are located in the ancient tea garden of Ganlongtan, Nanhua County, Yunnan Province (24°45′54″ N 100°50′54″ E–24°45′54″ N 100°50′55″ E), with an average slope of 5°. The soil is a typical yellow soil, in a garden facing southeast, and the plantations are laid out according to the contour lines, with tea trees of different ages intercropped to maximize soil utilization and sunlight exposure. The distance between the tea trees of different ages is approximately 200 m. Samples were collected on the morning of 13 April 2024, at 10:00 a.m., using a single-tree, five-point sampling method. All the sampled tea plants were identified as *Camellia sinensis* var. assamica (J. W. Mast.) Kitam. The planting ages of the tea trees were categorized into the following stages: approximately 30 years (GA), approximately 60 years (GB), approximately 90 years (GC), approximately 120 years (GD), approximately 180 years (GE), and approximately 200 years (GF) (Figure 1). The sampling was conducted using a single-tree, five-point sampling method to collect fresh tea leaf samples, comprising one bud and two leaves, from tea trees of different planting ages; approximately 200 g per sample was collected. Simultaneously, five-point sampling was applied to collect 200 g of tea rhizosphere soil near the tree roots for mixing and air-drying treatment. Additionally, within the projection range of the sampled plant's canopy, we carefully excavated the soil surrounding the tea tree roots using a small shovel, collected approximately 20 g of rhizosphere soil at a depth of around 20 cm, and rapidly froze it in liquid nitrogen, then stored it at −80 °C. The fresh tea leaf samples were processed in a microwave oven at medium–high power for 70 s to deactivate the enzymes and stop oxidation. The leaves, after microwave treatment, were placed in an oven and dried at 80 °C until completely dry to obtain the tea leaf samples.

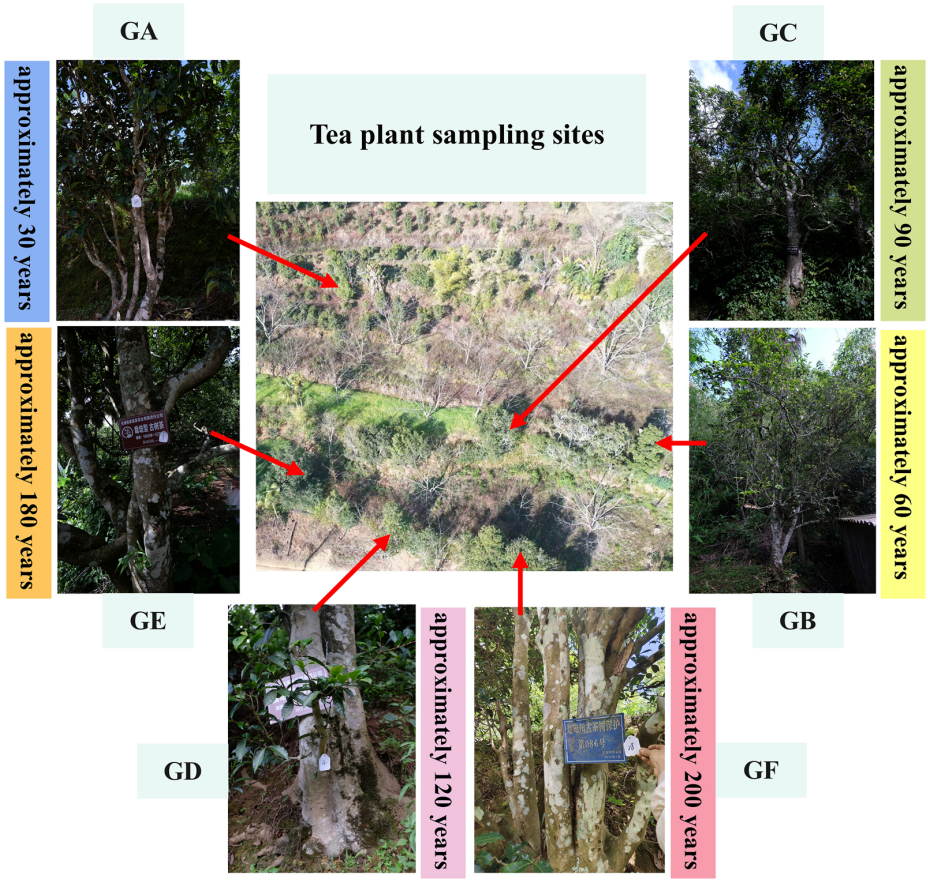

Figure 1. Tea plants at different planting ages and sampling locations.

2.2. Determination of Tea and Soil Physicochemical Indices

The tea water extracts (WEs) were determined according to GB/T 8305-2013 [25]. The content of tea polyphenol (TP) was measured using the Folin–Ciocalteu method [26]. The content of free amino acid (AA) was determined by ninhydrin chromogenic spectrophotometry [27]. The tea leaf powder (0.2 g) was wetted with 1 mL of deionized water and mixed with 5 mL of concentrated sulfuric acid. After setting overnight, the samples were then digested using H_2SO_4–H_2O_2 until the solutions were transparent. Macronutrients, namely, nitrogen (N), phosphorus (P), and potassium (K), were subsequently measured using a flow autoanalyzer (Skalar, Breda, Holland). A digest solution without plant samples was included as control [28]. All the measurements were repeated three times to ensure accuracy and reliability.

The residual rhizosphere soil samples were first air-dried naturally, then the stones and plant roots were removed. The soil was ground and sifted through a 100-mesh (0.15 mm) sieve to ensure the uniformity of the sample for subsequent chemical analyses. A 5 g subsample of soil was used to determine the soil pH using a pH meter in water; a 0.1 g subsample was utilized for the determination of total nitrogen (TN) using the concentrated sulfuric acid–hydrogen peroxide digestion–Kjeldahl nitrogen method. For the determination of total phosphorus (TP), a 0.25 g subsample was used, employing the sulfuric acid–hydrogen peroxide digestion–molybdenum antimonate colorimetric method. To determine total potassium (TK), a 0.25 g subsample was taken and analyzed using the concentrated sulfuric acid–hydrogen peroxide digestion–flame photometry method. For the measurement of alkali-hydrolyzable nitrogen (AN), a 2 g subsample was weighed and determined by the 1 mol/L NaOH alkaline extraction diffusion method. A 0.1 g subsample was taken for the determination of soil organic matter (SOM) using the oxidative thermal potassium dichromate oxidation–colorimetric method. For the determination of available phosphorus (AP), a 2.5 g subsample was used, applying the sodium bicarbonate solution extraction–molybdenum antimonate colorimetric method. To determine the available potassium (AK), a 5 g subsample was weighed and analyzed using the molybdenum antimonate–ascorbic acid colorimetric method [29]. All the measurements were repeated three times to ensure their accuracy and reliability. All the chemicals were purchased from Kunming Jin'ang Technology Co., Ltd. (Kunming, Yunnan Province, China).

2.3. Soil Microorganism Analysis

High-Throughput Sequencing Analysis of16S/18S rDNA

In this study, we utilized the TGuide S96 Magnetic Soil and Fecal DNA Kit (Tiangen Biotech, Beijing, China) for the extraction of genomic DNA from the rhizosphere soil of tea plants. The kit employs a distinctive decolorizing buffer system and magnetic bead technology to efficiently remove humic acids from the soil and lyse complex sample matrices, ensuring the integrity and purity of the extracted DNA. The extraction process encompasses sample lysis, magnetic bead purification, protein removal, and washing, followed by the elution of the DNA. This method is straightforward and rapid, suitable for a variety of soil environments and fecal samples, and the extracted DNA is directly applicable for molecular biology experiments such as PCR. The quality and quantity of the extracted DNA were examined using electrophoresis on a 1.8% agarose gel, and the DNA's concentration and purity were determined with a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, MA, USA). The hypervariable regions, V3-V4, of the bacterial 16S rRNA gene were amplified with the following primer pairs: 338F:5′-ACTCCTACGGGAGGCAGCA-3′; 806R:5′-GGACTACHVGGGTWTCTAAT-3′. For the fungal community, the ITS2 region of ITS gene was amplified using the primer pairs ITS2F:5′-GCATCGATGAAGAACGCAGC-3′ and ITS2R:5′-TCCTCCGCTTATTGTATGC-3′. Both the forward and reverse 16S/18S primers were tailed with sample-specific Illumina index sequences to allow for deep sequencing. The PCR was performed in a total reaction volume of 10 μL comprising the following: DNA template 5–50 ng, forward primer (10 μM) 0.3 μL, reverse primer (10 μM) 0.3 μL, KOD FX Neo Buffer 5 μL, dNTP (2 mM each)

2 µL, KOD FX Neo 0.2 µL, and, finally, ddH2O up to 10 µL. The process involved initial denaturation at 95 °C for 5 min, followed by 20 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, as well as a final step at 72 °C for 7 min. The amplified products were purified with an Omega DNA purification kit (Omega Inc., Norcross, GA, USA) and quantified using Qsep-400 (BiOptic, New Taipei City, Taiwan). The amplicon library was paired-end sequenced (2 × 250) on an Illumina Novaseq6000 (Beijing Biomarker Technologies, Beijing, China).

2.4. Bioinformatic Analysis

Operational Taxonomic Units (OTU) Cluster

The bioinformatics analysis of this study was performed with the aid of the BMKCloud (http://www.biocloud.net/ (accessed on 20 April 2024)). According to the quality of a single nucleotide, the raw data was primarily filtered by Trimmomatic (Version 0.33) [30]. The identification and removal of primer sequences was processed by Cutadapt (Version 1.9.1) [31]. The PE reads obtained from the previous steps were assembled by USEARCH (Version 10) [32], and this was followed by chimera removal using UCHIME (Version 8.1) [33]. The high-quality reads generated from the above steps were used in the following analysis. Sequences with a similarity of >97% were clustered into the same operational taxonomic unit (OTU) by USEARCH (v10), and the OTUs of less than 2 in all the samples were filtered.

The taxonomic annotation of the OTUs was performed using the Naive Bayes classifier within QIIME2, utilizing both the SILVA database (release Version 138.1) and the UNITE database (Version 8.0) [34] for the taxonomic classification of bacteria and fungi, with a confidence threshold of 70%. The alpha diversity was calculated and displayed using both QIIME2 (Version 2020.6.0) and R (Version 4.3.2) software. The beta diversity was determined using QIIME to assess the similarity of microbial communities across different samples. A principal coordinate analysis (PCoA), heatmaps, UPGMA, and non-metric multidimensional scaling (NMDS) were employed to analyze the beta diversity. Additionally, we utilized linear discriminant analysis (LDA) effect size (LEfSe) to test for significant taxonomic differences among groups [35]. A logarithmic LDA score of 4.0 was set as the threshold for discriminative features. To explore the dissimilarities in the microbiome across different factors, we performed a redundancy analysis (RDA) in R using the "vegan" package (Version 2.6-8).

3. Results

3.1. Changes in the Physicochemical Properties of Tea Tree Cultivation Soil with Different Planting Ages

The chemical properties of the tea tree rhizosphere soil exhibited significant changes with the increase in the tea tree planting age. As shown in Figure 2, the soil pH showed a decreasing trend from the GA (approximately 30 years) to the GD (approximately 120 years) stage, followed by an increasing trend, and returned to levels close to those of the GA stage by the GF (approximately 200 years) stage. Additionally, the TP and AP in the soil were significantly lower at the GE (approximately 180 years) stage compared to the GA stage, but a significant rebound was observed from the GE to the GF stage. The AN showed a remarkable increase at the GD stage, while the TN significantly increased at the GC (approximately 90 years) and GE stages. The TK and AK contents in the soil also displayed a similar trend, decreasing initially and then increasing, with the turning point occurring at the GD stage. The SOM contents followed a similar pattern, decreasing and then increasing, with the inflection point at the GD stage. These results indicate that the GD stage is a critical period in the growth process of tea trees, where changes in soil chemical properties may significantly affect tree growth and tea quality.

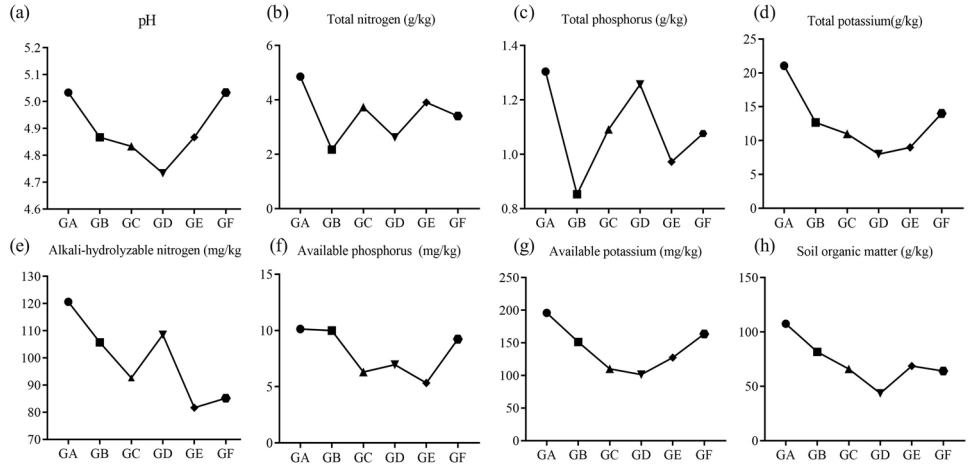

Figure 2. Variations in the soil pH (**a**), total nitrogen (**b**), total phosphorus (**c**), total potassium (**d**), alkali-hydrolyzable nitrogen (**e**), available phosphorus (**f**), available potassium (**g**), and soil organic matter (**h**) in tea tree cultivation soil with different planting ages.

3.2. Changes in Tea Leaf Chemical Compositions Across Different Planting Ages

The study observed a close relationship between the changes in N, P, and K contents in the tea tree leaves and the dynamics of tea quality components, which serve as important indicators of tea tree growth conditions. Figure 3 illustrates that the WE significantly increased at the GB (approximately 60 years) stage and then significantly decreased at the GE (approximately 180 years) stage; the TP significantly increased from the GA (approximately 30 years) to GB stage, but showed a decreasing trend from the GB to GD stage, and started to increase again after the GD (approximately 120 years) stage; the AA significantly increased at the GC (approximately 90 years) stage, marking this stage as a turning point for quality component changes. The N, P, and K contents in the leaves showed a decreasing trend from the GA to GB stage, then slowly rebounded at the GD stage, and tended to stabilize from the GE to GF (approximately 200 years) stage, especially with P and K significantly increasing at the GF stage, while nitrogen showed an opposing trend. These observations indicate that the GD stage is a critical period in the growth process of tea trees for changes in the tea leaf chemical composition.

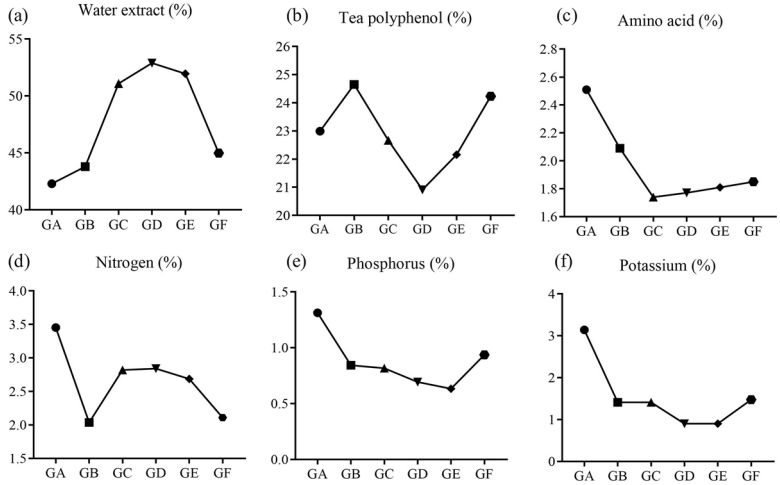

Figure 3. Changes in the water extract (WE) (**a**), tea polyphenol (TP) (**b**), amino acid (AA) (**c**), nitrogen (N) (**d**), phosphorus (P) (**e**), and potassium (K) (**f**) content in tea leaves from tea trees with different planting ages.

3.3. Influence of Tea Tree Planting Age on Soil Microbial Diversity

The alpha diversity analysis indicated significant effects of the tea tree planting age on soil fungal communities. As shown in Figure 4a, fungal community diversity varied significantly among the GB (approximately 60 years), GC (approximately 90 years), and GD (approximately 120 years) growth stages ($p < 0.05$), suggesting that an increasing tea tree planting age promotes increased fungal community diversity. The principal coordinate analysis (PCoA) further confirmed this, demonstrating distinct separations in soil fungal communities associated with tea trees of different planting ages, particularly during the GB, GC, and GD stages (Figure 4c). For soil bacterial communities, the alpha diversity analysis also revealed significant increases in bacterial community diversity with the increasing tea tree planting age (Figure 4b). Bacterial community diversity at the six distinct growth stages all exhibited significant differences, highlighting the substantial impact of tea tree planting age on the soil bacterial community structure. The PCoA analysis showed that bacterial communities at the GB and GD stages were more similar, possibly reflecting the specificity of tea tree–soil–microorganism interactions during these phases (Figure 4d).

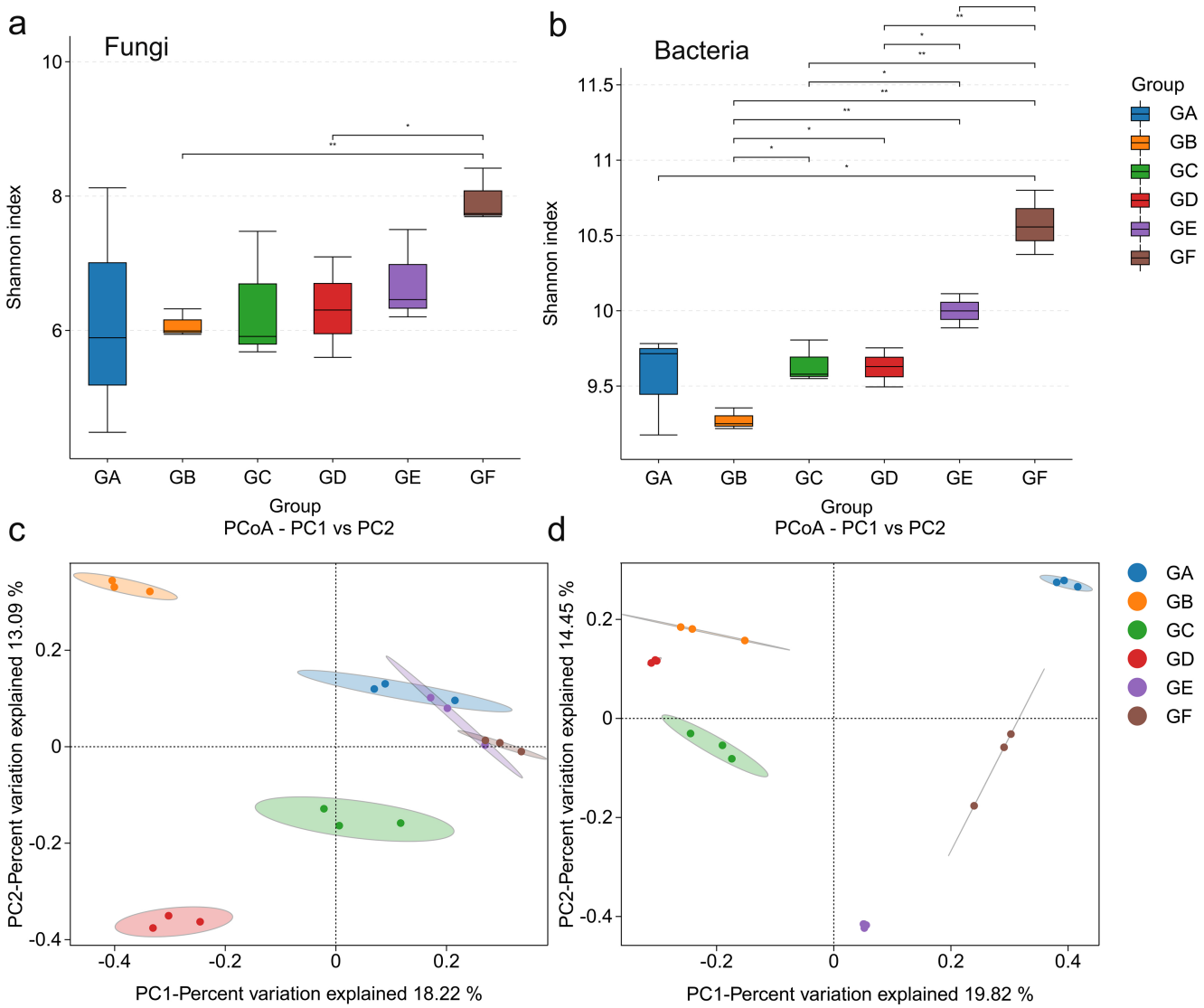

Figure 4. The impact of the tea tree planting age on the alpha diversity of soil fungal and bacterial communities (**a**,**b**), and the PCoA showing the distribution of soil fungal and bacterial communities across the different planting ages (**c**,**d**); * indicates significant difference, $p < 0.05$, ** indicates significant difference, $p < 0.01$, no marking indicates no difference.

The dendrogram constructed using the Unweighted Pair Group Method with the Arithmetic Mean (UPGMA) method revealed clustering relationships within the soil fungal and bacterial communities at various growth stages. As shown in Figure 5, the microbial communities at the GB (approximately 60 years) and GD (approximately 120 years) stages were grouped together, indicating a high similarity in soil microbial composition during these phases. Conversely, samples from the GA (approximately 30 years) and GF (approximately 200 years) stages formed distinct clusters, suggesting shared characteristics in microbial community structure at these stages. Notably, the sample at the GE (approximately 180 years) stage formed an isolated cluster in the dendrogram, indicating significant differences in the microbial community structure compared to the other stages.

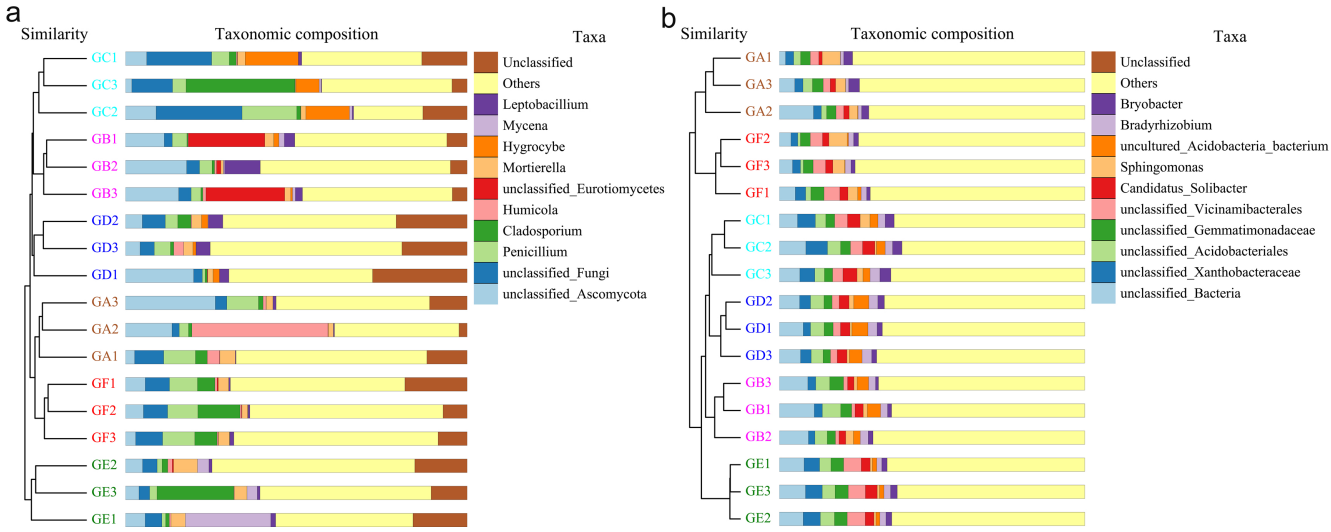

Figure 5. Dendrogram of soil fungal and bacterial community structures, based on the UPGMA method, with (**a**) for fungi and (**b**) for bacteria.

3.4. Analysis of Key Microbial Communities in Tea Garden Soil with Different Planting Ages

With the increase in the tea tree planting age, significant changes occurred in the composition of microbial communities in the tea garden soil. Specifically, among tea garden soil fungi (Figure 6a), Ascomycota and Basidiomycota were the dominant fungal phyla, with their abundance closely tied to the tea tree planting age. The proportion of Ascomycota across the six growth stages was 85.47%, 65.05%, 56.24%, 80.52%, 56.16%, and 76.77%, respectively. This trend showed an initial decrease followed by an increase, with the GD (approximately 120 years) stage marking the turning point, after which Ascomycota abundance gradually returned to levels near those of the GA (approximately 30 years) stage. In contrast, the Basidiomycota proportions were 5.45%, 27.94%, 20.66%, 8.10%, 27.80%, and 9.54% across the six stages, respectively, with its abundance initially increasing then decreasing, also peaking at the GD stage. These results indicate that the tea tree planting age significantly influences the abundance and composition of dominant fungal populations in the soil, with the GD stage being a critical period for changes in the microbial community structure.

Regarding the tea garden soil bacteria (Figure 6b), Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, and Bacteroidota were the dominant bacterial phyla. Proteobacteria relative abundance across the six growth stages was 34.88%, 21.79%, 28.35%, 25.71%, 28.10%, and 30.32%, respectively. This trend showed an initial decrease followed by an increase, with the GD stage marking the turning point, after which Proteobacteria abundance gradually returned to levels near those of the GA stage. Acidobacteriota relative abundance across the six stages was 16.02%, 23.58%, 27.17%, 28.81%, 26.20%, and 18.06%, respectively, with its trend initially increasing then decreasing, also peaking at the GD stage. Notably, from the GA to the GF (approximately 200 years) stage, Actinobacteriota and Chloroflexi abundance showed fluctuating trends, while Bacteroidota abundance remained

relatively stable from the GA to GF stages. These changes suggest that the tea tree planting age significantly impacts soil bacterial community diversity and composition, with the GD stage being a critical period for changes in bacterial community structure.

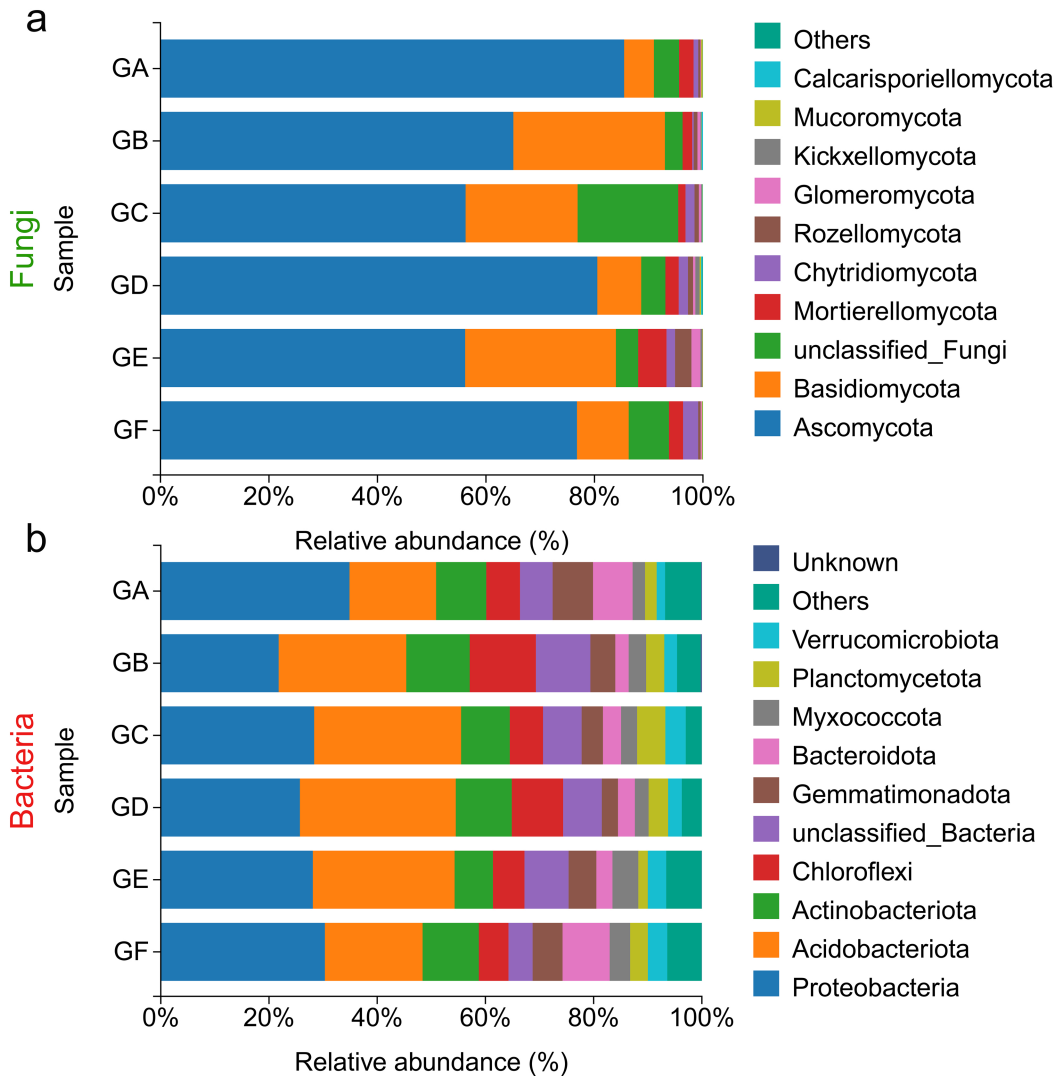

Figure 6. Distribution of the top 10 fungal (**a**) and bacterial (**b**) phyla in tea garden soil at different planting ages.

The LEfSe analysis was conducted to further understand the specific impact of the tea tree planting age on soil microbial communities (Figure 7a). This analysis revealed the abundance changes of characteristic microbes (at the genus level) in tea garden soil across the different planting ages and generated the corresponding evolutionary cladograms. In the bacterial community, Gemmatimonas significantly contributed to the microbial communities at the GA (approximately 30 years) stage; unclassified Acidobacteriales, an uncultured forest soil bacterium, and unclassified Chloroflexi significantly contributed to the GB stage; unclassified Gemmataceae, Candidatus Solibacter, and unclassified Xanthobacteraceae significantly contributed to the GC (approximately 90 years) stage; uncultured Acidobacteria bacterium, Catenulispora, and uncultured eubacterium WD298 significantly contributed to the GD (approximately 120 years) stage; unclassified Vicinamibacterales significantly contributed to the GE (approximately 200 years) stage; and Sphingomonas significantly contributed to the GF (approximately 180 years) stage.

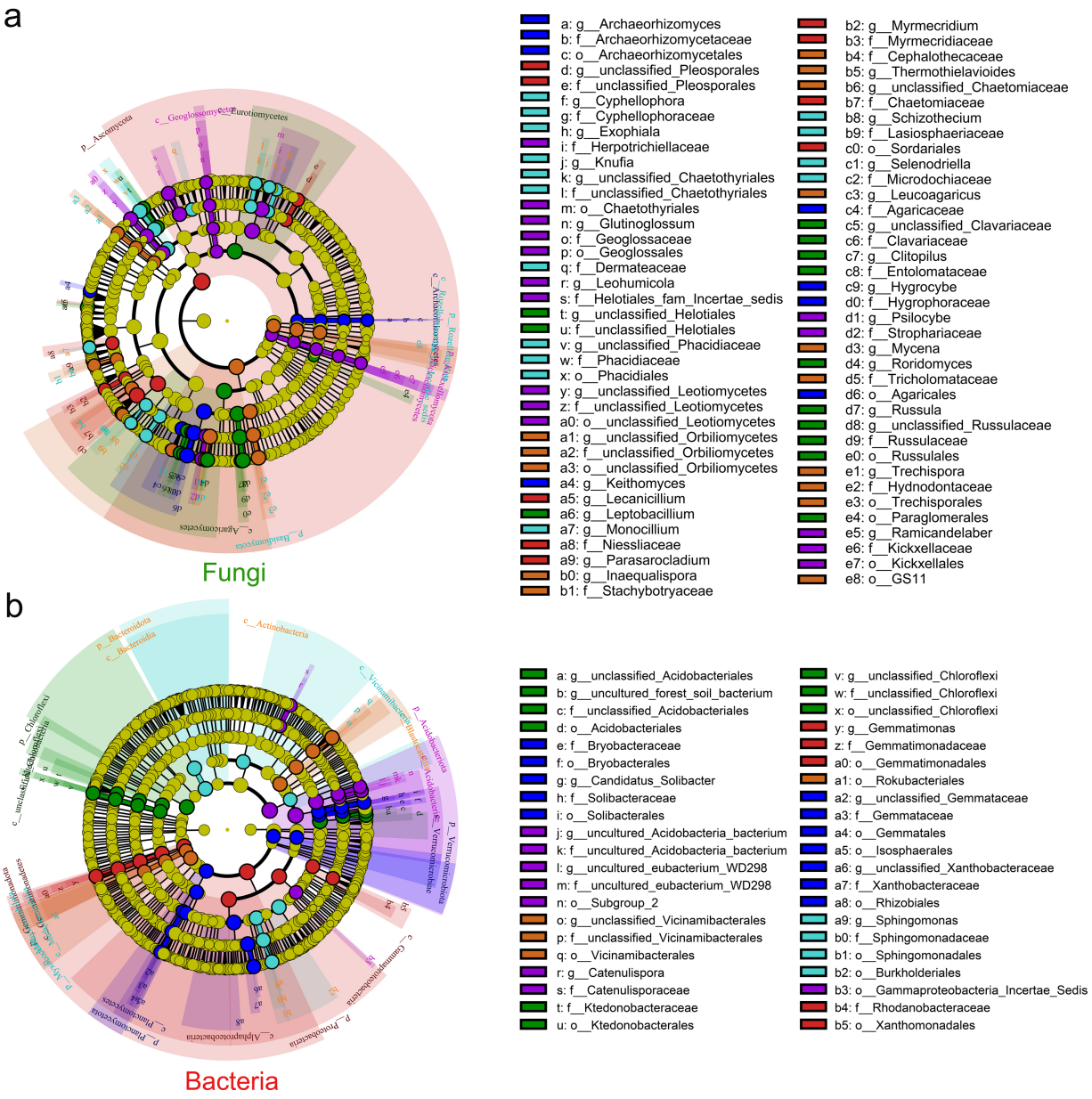

Figure 7. LEfSe analysis depicting the evolutionary cladogram of the abundance changes of characteristic microbial fungi (**a**) and bacteria (**b**) in tea garden soil across the different planting ages.

In the fungal community(Figure 7b), genera such as Lecanicillium, Parasarocladium, and Myrmecridium significantly influenced the microbial community structure at the GA stage; Leptobacillium, unclassified Helotiales, Clitopilus, unclassified Clavariaceae, unclassified Russulaceae, Roridomyces, and Russula significantly influenced the GB stage; Archaeorhizomyces, Keithomyces, and Hygrocybe significantly influenced the GC stage; Glutinoglossum, Leohumicola, unclassified Leotiomycetes, Psilocybe, and Ramicadelaber significantly influenced the GD stage; Inacqualispora, Thermothielavioides, unclassified Orbiliomycetes, Leucoagaricus, unclassified Chaetomiaceae, Mycena, and Trechispora significantly influenced the GE stage; and Cyphellophora, Exophiala, Knufia, unclassified Chaetothyriales, Monocillium, Schizothecium, and Selenodriella significantly influenced the GF stage.

3.5. Functional Analysis of Key Microbial Communities in Tea Garden Soil with Different Planting Ages

Utilizing the FUNGuild database, a functional prediction of the soil fungal communities was conducted (Figure 8a). The results indicated that the Wood Saprotrophs were most abundant in the GA (approximately 30 years) (23.08%) and GE (approximately 180 years) stages (27.75%), with a significant decline observed in the intervening stages. Plant pathogens were relatively more abundant in the GC (approximately 90 years), GE, and GF (approximately 200 years) stages, constituting 12.48%, 17.79%, and 20.79% respectively. The Ectomycorrhizal guild was most prominent in the GB (approximately 60 years) stage, accounting for 28.49%, and was less than 1% in all other stages. Leaf Saprotrophs peaked in the GE stage, making up 10.47% of the community. Using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database, the soil bacteria were functionally annotated (Figure 7b). The top 10 bacterial functions were displayed, five of which are related to soil nutrient cycling, including aerobic chemoheterotrophy, nitrogen fixation, ureolysis, photoheterotrophy, and cellulolysis. Aerobic chemoheterotrophy was the most represented function in the top 10, maintaining a proportion of over 30% from the GA to the GD (approximately 120 years) stages, after which it showed a declining trend. Nitrogenen fixation increased from the GA to the GD stage, reaching its maximum proportion (9.44%) at the GD stage, then declined. Ureolysis began to decrease from the GB stage and recovered to the levels of the GA stage by the GF stage.

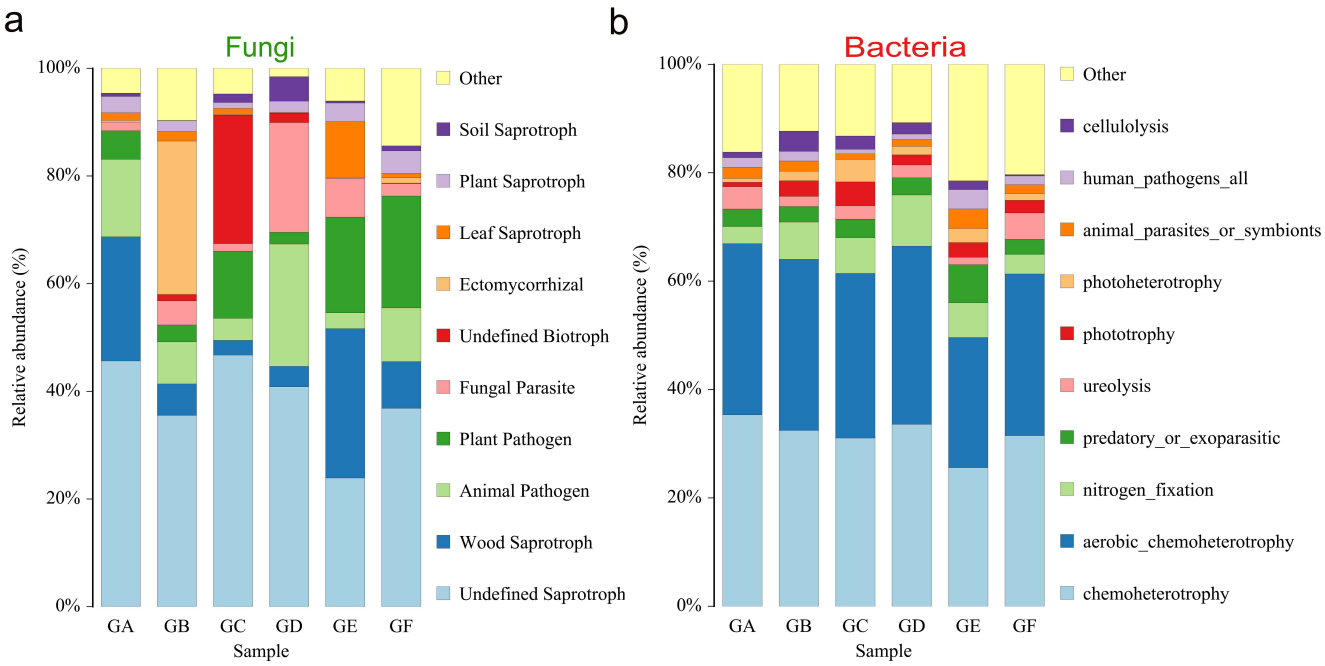

Figure 8. Functional analysis of key microbial communities in tea garden soil with different tea tree planting ages, with (**a**) representing fungi and (**b**) representing bacteria.

3.6. Correlation Analysis Between Soil Microbial Community Structure and Soil Properties

The RDA was employed to investigate the relationship between soil microbial communities and environmental factors. The results showed that in the soil fungal community (Figure 9a), the TP, SOM, TN, and TK exhibited strong correlations with the structure of the soil fungal microbial community, particularly in the GA stage samples. For the GB, GC, GD, and GE stages, the microbial communities were primarily influenced by Hygrocybe, Mycena, Leptobacillium, and Mortierella, while in the GF stage, Cladosporium had the most significant impact. Additionally, Humicola and Penicillium were closely associated with the formation of soil nutrient components. In the bacterial community, the pH, AN, SOM, and TP showed strong correlations with the structure of the soil bacterial microbial community (Figure 9b), with the GA stage samples again showing the most significant

correlations. Bradyrhizobium and Candidatus Solibacter had strong correlations with the bacterial communities in the GB, GC, GD, and GE stages, while unclassified Gemmatimonadaceae had the most significant impact in the GF stage. Bryobacter and Sphingomonas were closely related to soil nutrient formation, likely due to their roles in the decomposition of soil organic matter and nutrient cycling.

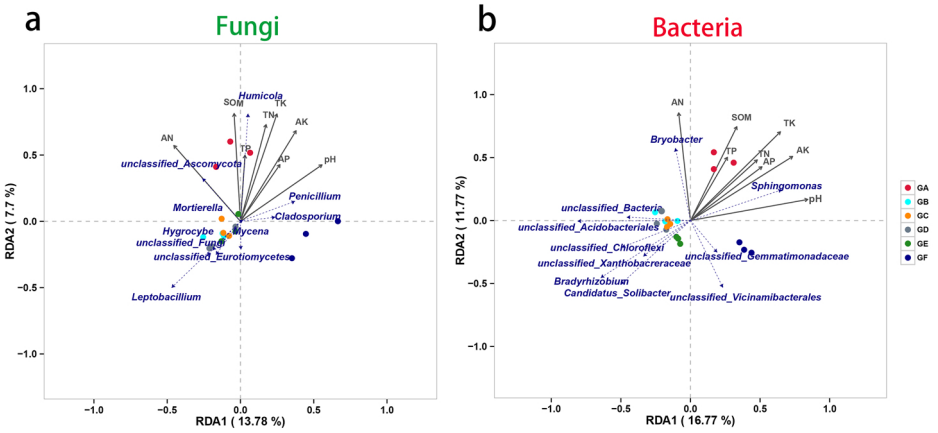

Figure 9. RDA of the relationship between soil microbial community structure and soil properties, with (**a**) depicting fungi and (**b**) depicting bacteria.

4. Discussion

Soil serves as the medium for tea tree growth, and its fertility directly influences the tea yield and quality. Our study found that as the planting age of tea trees increased, the soil pH exhibited a decreasing trend from the GA (approximately 30 years) to the GD (approximately 120 years) stage, which is consistent with previous research [36]. This decrease in pH is attributed to the accumulation of organic acids such as oxalic, citric, and malic acids from tea tree roots. However, from the GD to the GF (approximately 200 years) stage, the soil pH showed an increasing trend, potentially associated with the increased diversity of rhizosphere microorganisms. Additionally, as the planting age of the tea garden increased, the soil TK, SOM, AP, AN, and AK initially decreased and then began to rebound at the GC (approximately 90 years) or GD stage. This phenomenon may be linked to the natural restoration mechanism of soil fertility. The accumulation of nutrient elements and chemical components changes with the aging of tea trees, particularly affecting the metabolism of phenolic compounds and amino acids in tea leaves [37,38]. In our study, the TP, AA, N, P, and K contents in tea leaves showed significant variations, with a pattern of initial increase followed by a decrease and then another increase, which is closely associated with changes in soil microbial activity. This indicates that soil microorganisms play a pivotal role in the nutrient cycle of tea trees and the formation of tea quality.

The soil fungi play a crucial role in the nutrient cycling of tea tree soil, especially in the cycles of carbon, nitrogen, and phosphorus. They not only promote the decomposition of organic matter to provide nutrients for tea trees but also improve the soil structure and enhance soil aeration and water retention [39]. Our study results show that with the increase in tea tree planting age, the Shannon index indicates that fungal community diversity also increases; as the age of tea tree cultivation increases, significant changes in soil fungal diversity occur. Particularly during the GA stage, the fungal diversity, along with the standard deviation, encompasses all other diversity values, which may be related to changes in soil physicochemical properties, plant–microbe interactions, environmental stress, and adaptability. The combined effects of these factors may lead to differences in the fungal diversity in the rhizosphere soil of tea trees at different age stages. Furthermore, although the diversity values of the GA stage statistically exceed those of other stages, this does not exclude the possibility that tea trees at other age stages may exhibit unique microbial community structures under specific environmental conditions. Therefore, our study

emphasizes the importance of conducting in-depth research on the rhizosphere microbial communities of tea trees at different age stages. Among these fungi, Archaeorhizomyces is a genus that has been isolated from the root tips of plants [40], and Russula, widely distributed geographically and ecologically, forms ectomycorrhizal relationships with various plants, aiding in nutrient absorption from the soil [41]. Leptobacillium species are important decomposers in soil; they break down plant residues, animal remains, and other organic matter, participating in soil nutrient cycling. Certain Leptobacillium species can fix atmospheric nitrogen, converting it into a form usable by plants, which is crucial for plant growth [42]. Mortierella species can decompose soil oils and lipids, aiding in soil purification and nutrient cycling. Some Mortierella species can inhibit the growth of plant pathogens in the soil, enhancing plant disease resistance [43].

The RDA results indicate that in the GB (approximately 60 years), GC, GD, and GE (approximately 180 years) stages, genera such as Hygrocybe Mycena, Humicola, and Penicillium can decompose organic matter in the soil, converting it into nutrients that plants can absorb, thereby promoting soil nutrient cycling. They can also improve soil structure; their hyphal growth aids in enhancing soil aeration and water retention [44,45]. Humicola species help plants absorb phosphorus and nitrogen from the soil. Some Humicola species can degrade toxic substances in the soil, such as heavy metals and organic pollutants, aiding in soil remediation [46]. In the GF stage, Cladosporium species are important decomposers in the soil; they can break down plant residues, dead branches, and other organic matter, converting organic matter into a form that plants can absorb, promoting soil nutrient cycling. Their hyphal growth and expansion improve the soil structure, increasing soil aeration and water retention, positively affecting the physical properties of the soil [47].

The diversity of soil bacterial communities affects the biogeochemical cycling of soil nutrients and plays a crucial role in maintaining sustainable agriculture [48]. Therefore, this study used high-throughput sequencing methods to investigate the dynamic changes in bacterial communities in the rhizosphere of tea trees of different ages. The microbial diversity analysis revealed significant differences in the composition of soil bacterial communities across the tea garden time series, consistent with previous research findings [49]. Further analysis of the soil bacterial community revealed that with the increasing planting age, the abundance of Acidobacteriota and Proteobacteria phyla increased, including genera such as Candidatus Solibacter, Catenulispora, and Sphingomonas, which participate in promoting organic matter decomposition [50,51]. Additionally, Sphingomonas has been shown to produce plant growth regulatory substances, such as plant hormones, promoting plant growth and participating in the transformation and cycling of soil nutrients like nitrogen and phosphorus. Some Sphingomonas species have phosphorus-solubilizing effects, aiding the plant absorption of soil phosphorus [52]. Gemmatimonadaceae primarily maintains soil fertility by decomposing organic matter and releasing nutrient elements, forming symbiotic relationships with plant roots, promoting plant growth [53]. Bradyrhizobium is a key soil bacterial genus that forms symbiotic relationships with leguminous plants, enhancing plant nitrogen availability through nitrogen fixation and promoting plant growth. It also aids in soil phosphorus utilization, improves soil structure, increases soil fertility, and inhibits plant diseases, adapting to various soil environments, participating in soil bioremediation processes, and supporting ecosystem services and biodiversity [54]. Bryobacter's ecological functions are significant for regulating soil microbial communities, improving soil quality, and enhancing crop yields [55]. Our research found that starting from the GC stage, the abundance of pathogens significantly increased, which may have a significant impact on the composition and function of the soil microbiome. This phenomenon may be related to changes in soil nutrients, variations in plant root exudates, and plant responses to environmental stress.

The planting age of tea trees significantly affects tea quality, and this impact is closely related to the structure and function of soil microbial communities [23,56]. Our study found that as the planting age of tea trees increases, the changes in soil microbial diversity and key microbial populations may directly affect the chemical composition and sensory

quality of the tea. The abundance changes of specific fungi and bacteria may correlate with the accumulation patterns of quality-related components such as phenolic compounds, amino acids, and tea polyphenols in tea leaves [39]. Furthermore, microbial communities indirectly regulate tea quality formation by participating in soil nutrient cycling, affecting soil fertility and plant nutritional status [57]. These findings provide new insights into the impact of tea tree–soil–microbe interactions on tea quality.

5. Conclusions

This study analyzed the effects of the tea tree planting age on the structure and function of soil microbial communities and the consequent impact on the tea quality. The findings reveal significant changes in soil's chemical properties, such as the pH, TP, AP, AN, TN, TK, AK, and SOM, with the increasing tea tree planting age. Notably, around the 120 years mark, these soil chemical properties reach a critical turning point, indicating this stage as a pivotal period for soil fertility changes during the growth cycle of tea trees. Additionally, the contents of WE, TP, AA, and the levels of N, P, and K in tea leaves also exhibit variations with the extension of tea tree planting age. The increase in tea tree planting age promotes the diversity of fungal and bacterial communities, and the key microbial community analysis highlighted the shifts in the dominant microbial populations in the soil at the different growth stages. Genera such as Hygrocybe Mycena, Humicola, Penicillium (fungi), Bradyrhizobium, Candidatus Solibacter, Sphingomonas, and Gemmatimonadaceae (bacteria) play crucial roles in soil nutrient cycling and plant growth, for instance, by facilitating the decomposition of organic matter, enhancing plant growth, and participating in the transformation and cycling of soil nutrients like nitrogen and phosphorus, significantly influencing the nutrient status and growth vigor of tea trees. Furthermore, the abundance of bacteria involved in aerobic chemoheterotrophy and nitrogen fixation tends to gradually decrease with the increase in planting age. This study emphasizes the indirect regulation of tea quality formation through the influence of the tea tree planting age on the structure and function of soil microbial communities, offering new insights into the impact of tea tree–soil–microbe interactions on tea quality.

Author Contributions: H.W. conceived the overall design and conceptualization of the study, drafted the manuscript, and completed the final revision; conceptualization, methodology, T.W. and W.Y.; investigation, resources, software, L.C.; software, formal analysis, methodology, validation, H.L. and X.D.; C.W. and W.L. managed the manuscript editing and reviewed revisions; W.H. and B.W. provided financial support and project management and reviewed revisions. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Science and Technology Mission of Smart Tea Industry in Menghai County, Yunnan Province (202304BI090013); the Yunnan Tea Industry Artificial Intelligence and Big Data Application Innovation Team (202405AS350025); and the research and development and demonstration project of data sensing technology and equipment for high-altitude mountain smart agriculture (202302AEO9002001).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no potential conflicts of interest.

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