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Application of Wheat Straw Compost Mixed with Chemical Fertilizer Regulates Soil Bacterial Community Diversity in Tea (*Camellia sinensis*) Plantation

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Abstract: Biofertilizers have been suggested as alternatives to synthetic fertilizers, which could reduce soil degradation brought on by excessive chemical fertilization and have an impact on the bacterial diversity and community in the soil. The diversity and community of soil bacteria in tea plantations treated with wheat straw compost have, however, received relatively little attention. In this research, a two-year field trial was run to examine the effects of applying wheat straw compost on the characteristics of the soil and the quality of the tea. We also used high-throughput sequencing to investigate the response of the soil bacterial community, and Spearman's rank correlation was used to estimate the relationship between the soil bacterial community, soil characteristics, and tea quality. It was noticed that applying chemical fertilizer along with compost increased the fertility of the soil and the quality of the tea. Based on a two-year thorough data analysis, the T4 treatment (compost fertilizers 15,000 kg ha^{-1} + chemical fertilizers 1050 kg ha^{-1}, chemical fertilizer reduction 30%) was determined to be the best group. The diversity and community makeup of soil bacteria were impacted by fertilization management. After the initial compost replacement, soils with compost had a greater bacterial richness than soils with inorganic fertilizers. After the second compost substitution, PCoA analysis revealed that compost fertilizer could be easily differentiated from chemical fertilizer. In 2019, Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Patescibacteria were the most prevalent bacterial phyla. In 2020, Firmicutes and Chloroflexi overtook Bacteroidetes and Patescibacteria as the two major bacterial phyla. In addition to increasing the diversity of soil bacteria and having an impact on the bacterial population, the application of wheat straw compost mixed with chemical fertilizers can also control the soil's characteristics and the quality of the tea produced in tea plantations. So, as a fertilization way with less environmental impact, wheat straw compost fertilization can be used in tea plantations.

Keywords: *Camellia sinensis* (L.) O. Kuntze; wheat straw compost; tea plantation soil; bacterial community

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1. Introduction

The tea plant (*Camellia sinensis* (L.) O. Kuntze) is significant commercial leaf crop, whose leaves can be made into different non-alcoholic beverages [1]. It originated in China and has been grown there for more than 2000 years [2]. China produces the majority (54.66% of the world's planted area and annual output) of tea, which is produced as fresh tea leaves (40.56%) [3]. Without a question, China has the world's largest tea planting area and highest tea yield. Tea has a number of health advantages, such as anti-oxidants, cancer prevention, cardiovascular safety, and allergy alleviation [4].

Fertilizer application has become a common practice in tea plantation to ensure crop productivity. On tea farms, fertilizer application rates are typically high in order to boost crop yields in China [5]. However, there is no assurance that increased fertilizer rates will lead to increased agricultural yields. In comparison, overuse of chemical fertilizers over a long period of time causes tea gardens' soil to become acidic and lose nutrients like P, K, and Mg [6]. Tea quality and production are both decreased by soil acidification, and tea leaves also become more contaminated with heavy metals [7,8]. Therefore, it is critical to create a sustainable plan to minimize the use of chemical fertilizers, boost crop production, and lessen the negative effects of overusing chemical fertilizer.

Bio-organic fertilizers are considered one of the most promising alternative sources [9,10]. Bio-organic fertilizers can influence the diversity and community makeup of the soil's bacterial population and reduce the soil acidification caused by the overuse of chemical fertilizers [11]. At present, various bio-organic fertilizers have been applicated to substitute chemical fertilizers partially or fully in tea plantation, including cow manure [12], organic fertilizer [13], biochar [14], biogas slurry [15], and crop straw compost [16]. In comparison to chemical fertilizers, research indicates that applying organic fertilization to tea plantations can increase soil fertility and productivity [17].

A vital component of soil function is the presence of extremely diverse microbial communities in soil [18]. Agro-ecosystem productivity and sustainable development were greatly enhanced by the contribution of soil bacteria [12,19–21]. Numerous studies have shown that the tea plantation's soil microbial diversity and community structure were significantly impacted by the fertilizer protocol [22]. The application of chemical nutrients over a long period of time changed the makeup of the soil's bacteria and reduced its metabolic activity, which led to a decline in the population of helpful bacteria [23]. On the other hand, organic fertilizer enhanced potential ecosystem function by broadening the diversity of soil microbes, changing the organization of networks, and increasing soil fertility [13,24,25].

Wheat straw is the second-most abundant agricultural residue in the world. Composting is a sustainable, effective, and ecofriendly approach to deal with wheat straw. It has previously been suggested that the treatment of tobacco soil with an application of combined chemical fertilizer/wheat straw compost led to improved soil chemical properties and increased soil organic matter, and resulted in changes in the microbial community composition. However, there has been little study on the bacterial communities in the soil of tea plantations using composted wheat straw. In this investigation, we conducted a two-year field trial (unfertilized, wheat straw compost alone, wheat straw compost mixed with chemical fertilizers, and chemical fertilizers alone) to examine the impacts of wheat straw compost on the diversity of the soil's microbial community in a tea plantation. A high-throughput sequencing method of 16S rRNA genes was used to analyze the diversity of the soil bacterial community, and the Spearman correlation was used to explore the relationships between the soil bacterial community and soil characteristics or tea quality. The objectives of the study were: (1) identify the differences in diversity and composition in soil bacteria with composted wheat straw, and (2) investigate the connections between the structure of the soil bacterial community and soil characteristics or tea quality. This research not only demonstrated the efficacy of using wheat straw compost to moderate the bacterial community in the soil, but it also established a crucial theoretical foundation for the sensible use of wheat straw compost fertilizer in tea plantations.

2. Materials and Methods

2.1. Experimental Site Description

An experimental plot was selected for the field experiments at Maling Spring Tea Industry Co., Ltd. tea plantation, Rizhao City, Shandong Province, China (35.48° N, 119.38° E). This region has a semi-humid monsoon climate and is in a warm temperate zone. The climate is mild, and four seasons are discernible. The annual average temperature is 14.1 °C, with a monthly mean temperature reaching a maximum of 30.0 °C in July and a minimum of −3.0 °C in January. Its annual average precipitation is 874 mm. The average annual sunshine duration is about 2533 h with annual frost-free 223 days. The soil type in the area is a light, sandy loam. The experimental site is dominated by tea fields. The tea tree variety was six-year-old 'Zhongcha 108′ with a row spacing of 1.5 m and plant spacing of 0.33 m. Prior to the establishment of the experiment, the soil background was as follows: alkali-hydrolysis nitrogen (AN), 130.4 mg kg^{-1}, available phosphorus (AP), 59.5 mg kg^{-1}; available potassium (AK), 179.75 mg kg^{-1}; pH 5.9; soil organic matter (SOM), 8.68 g kg^{-1}.

2.2. Fertilizer Preparation

Chemical fertilizers used in this experiment are commercially available and were purchased from Shindoo Chemi-industry Co., Ltd. (Chengdu, China). Chemical fertilizer with a ratio of N-P_2O_5-K_2O: 22-9-9 consists of total nutrient content $\geqslant$ 40% and contains both nitrate nitrogen and ammonium nitrogen (content of nitrate nitrogen $\geqslant$ 9%).

Wheat straw for composting is produced in Shandong Province, China. The organic carbon of wheat straw mainly consists of cellulose, hemicellulose, lignin, and mixed carbon of degradation products. The carbon-to-nitrogen ratio of wheat straw was 66:1. According to this ratio, urea was added to adjust the carbon-to-nitrogen ratio to 30:1. The aerobic compost material comprised a mixture of wheat straw, urea, brown sugar, and microbial inoculum. Overall, 1 ton of mashed wheat straw, 5 kg of urea, 2 kg of brown sugar, and 3 kg multispecies polymicrobial inoculum were mixed with an initial C/N ratio of approximately 30:1. The relative humidity was maintained at 60%. The microbial inoculum included *Bacillus subtilis*, *Bacillus pumilus*, *Saccharomyces*, and *Aspergillus oryzae*, microbial content $\geq 5 \times 10^8$ CFU g^{-1}. The composting process lasted approximately 35 days, and the piles were turned manually four times to keep the temperature below 60 °C and maintain aeration. Application of compost mixed with chemical fertilizers done at the time of the seed germination index (GI) $\geq$ 80 as per treatment plan. The microbial inoculum was obtained from Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education, Heilongjiang University (Harbin, China).

2.3. Experimental Design

The ratio of nitrogen–phosphorus–potassium (NPK) demand in tea plant was N:P_2O_5:K_2O = 3:1:1. The tea garden applied pure nitrogen (N) fertilizer 225–300 kg ha^{-1} in autumn. In practical production, however, tea farmers apply a large amount of chemical fertilizer, up to 1500–3000 kg ha^{-1}, in order to pursue high economic efficiency. Therefore, combined with the nitrogen fertilizer requirements of tea plants and the fertilization situation of tea farmers, the maximum fertilizer application rate was designed to be 1500 kg ha^{-1} (compound fertilizer, N-P-K content is 22-9-9), which was in line with the actual production.

This experiment consisted of seven treatments with a randomized complete block design. Each treatment had five replicates, and each plot was 135 m^2 (30 m × 4.5 m). The seven treatments were as follows: (1) CK, no fertilization, (2) T1, compost fertilizers 15,000 kg ha^{-1} alone, (3) T2, compost fertilizers 15,000 kg ha^{-1} plus chemical fertilizers 150 kg ha^{-1}, chemical fertilizer reduction 90%, (4) T3, compost fertilizers 15,000 kg ha^{-1} plus chemical fertilizers 600 kg ha^{-1}, chemical fertilizer reduction 60%, (5) T4, compost fertilizers 15,000 kg ha^{-1} plus chemical fertilizers 1050 kg ha^{-1}, chemical fertilizer reduction 30%, (6) T5, compost fertilizers 15,000 kg ha^{-1} plus chemical fertilizers 1275 kg ha^{-1}, chemical fertilizer reduction 15%, and (7) T6, chemical fertilizers 1500 kg ha^{-1} alone. Two fertilization treatments were performed sequentially in 18 October 2018 and 31 October 2019. The wheat straw compost was directly sown into rows, then the plots were turn-over ploughed by mini-tiller. The chemical fertilizers were applied to furrows.

2.4. Sample Collection

Tea leaves and soil samples were gathered on 21 May 2019 and 2020, respectively. The soil samples were taken at a depth of 20 cm from each experimental plot using a five-point sampling technique. The five soil replications, each weighing 20 g, were mixed uniformly to obtain a single thoroughly homogeneous sample for soil analysis. Each treatment had three samples. One portion of the soil samples was used for the analysis of soil nutrients, and the other portion was kept at −80 °C for total DNA extraction after being air-dried, ground, and sieved through a 2 mm sieve. The fresh tea leaves (50 g) were picked from each experimental plot under the different fertilization treatments. The tenderness of the material was a bud and two leaves. One sample was mixed with every five replicate samples, and then dried. The assay was repeated in triplicate for each treatment.

2.5. Determination of Physicochemical Properties of Soil

The soil's physicochemical characteristics were measured in accordance with National Standards of the People's Republic of China NY/T 1121.2-2006 for pH, NY/T 1121.6-2006 for soil organic matter, LY/T 1228-2015 for total nitrogen and alkali-hydrolysable nitrogen, NY/T 1121.7-2014 for available phosphate, and NY/T 889-2004 for available potassium, respectively.

2.6. Determination of Tea Quality Indices

In this trial, the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRI CAAS), Hangzhou, China, measured the contents of four chemical components of tea: tea polyphenols (TPs), free amino acids (FAA), caffeine, and water extractions (WEs). To assess the quality of tea, the ratios of polyphenols to free amino acids (TP/AA) were determined. The method of the National Standards of the People's Republic of China (GB/T 8313-2008, GB/T 8314-2013, GB/T 8305-2013, and GB/T 8312-2013, respectively) was used to identify TPs, FAA, WEs, and caffeine.

2.7. Soil DNA Extraction, PCR, and MiSeq Sequencing

The soil DNA was extracted using an OMEGA Soil DNA Kit. DNA was measured using a Nanodrop 2000 spectrophotometer, and the DNA integrity was evaluated using 1.2% agarose gel electrophoresis. Using the primers 338F and 806R (5′-ACTCCTACGGGAGGCAGCA-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′), the 16S rRNA variable V3-V4 region was amplified. PCR was performed with a final volume of 25 μL containing 5 μL 5 × reaction buffer, 5 μL 5 × GC buffer, 2 μL dNTP (2.5 mM), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 2 μL DNA template, 8.75 μL ddH_2O, and 0.25 μL Q5 DNA polymerase. Initial denaturation at 98 °C for 2 min was followed by 30 rounds of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, as well as a final extension at 72 °C for 5 min and a hold at 10 °C. After the PCR products were mixed and expanded several times for each sample, they were purified. Personalbio (Personal Biotechnology Co., Ltd., Shanghai, China) received the purified PCR result for double terminal sequencing analysis on the Illumina MiSeq platform.

2.8. Sequence Processing and Bioinformatic Analysis

The TruSeq Nano DNA LT Library Prep Kit from the Illumina Corporation was used to create the sequencing library. The double terminal sequences were paired using the Flash Programme (v1.2.7, http://ccb.jhu.edu/software/FLASH/ accessed on 19 March 2023) [26] after being mass-screened for overlap-ping bases. Finally, the connected sequence recognition was given to the corresponding sample based on the Index information corresponding to each sample, and the effective sequence of each sample was received. The sequence obtained above was combined, divided by 97% sequence similarity, and the sequence with the greatest abundance in each operational taxonomic unit (OTU) was chosen as the representative sequence of the OTU using QIIME software and the sequence alignment tool UCLUST [27]. The species annotation analysis was performed using the SILVA database (Release 115, http://www.arb-silva.de accessed on 19 March 2023) [28], and after that, the data for each sample were homogenized.

2.9. Statistical Analysis

To compare the differences in the physiochemical characteristics of soil, tea chemical components, and bacterial relative abundances (Tukey, $n = 3$), one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used in SPSS (SPSS, Inc., Chicago, IL, USA). If not specified otherwise, $p < 0.05$ was used as the significance level. The taxonomic alpha diversity indices, such as the Chao1 index and the Shannon index, were calculated using Mothur software (version 1.31.2, http://www.mothur.org/ accessed on 19 March 2023). The application of Venny (http://bioinfogp.cnb.csic.es/tools/venny/ accessed on 19 March 2023) was used to create the Venn diagram. Using the "pheatmap package" in the R Programme, Spearman's rank correlation analysis was used to examine any potential relationships between the relative abundance of bacterial phyla and soil characteristics.

3. Results

3.1. Effect of Fertilization Management on Tea Plantation Soil Physicochemical Properties

We examined the amounts of soil nutrients in seven treatments to assess the physicochemical properties of soils with various fertilizations (Table 1). Soil pH tended to decrease with an increasing chemical fertilizer, with lowest pH values being observed in T6 under chemical fertilization alone in both 2019 and 2020 (Table 1). During the first and second experimental years, the contents of soil organic matter (SOM), total nitrogen (TN), available N (AN), and available K(AK) increased with increasing dose of chemical fertilizers and reached a maximum value in T4 or T5 under the combination of chemical fertilizer and compost treatments, whereas these indices exhibited the lowest value under no fertilizers (CK), compost alone (T1), and chemical fertilizers alone (T6) treatments, except the contents of AK in T6 (Table 1). The contents of available P (AP) reached a maximum under compost alone (T1) and decreased with chemical fertilizers dosage in 2019. In contrast to the first fertilization treatment, the contents of AP increased with the dose of chemical fertilizers and reached a maximum value in T4 after the second fertilization treatment (Table 1).

Table 1. Physicochemical characteristics of soil samples under different fertilization treatments.

Year	Sample	Soil pH	SOM $(g \cdot kg^{-1})$	TN $(g \cdot kg^{-1})$	AN $(mg \cdot kg^{-1})$	AP $(mg \cdot kg^{-1})$	AK $(mg \cdot kg^{-1})$
2019	CK	5.90 ± 0.02^{ab}	8.96 ± 0.04^{f}	1.09 ± 0.02^{d}	132.67 ± 4.92^{f}	59.90 ± 1.26^{c}	177.33 ± 2.05^{e}
	T1	6.20 ± 0.16^{a}	9.71 ± 0.21^{e}	1.20 ± 0.03^{c}	151.33 ± 4.64^{e}	92.33 ± 0.38^{a}	181.33 ± 1.25^{e}
	T2	6.13 ± 0.17^{a}	10.27 ± 0.02^{d}	1.23 ± 0.04^{c}	176.33 ± 5.25^{cd}	80.83 ± 1.27^{b}	216.67 ± 4.64^{d}
	T3	6.10 ± 0.24^{a}	13.26 ± 0.03^{c}	1.49 ± 0.03^{b}	187.67 ± 4.99^{c}	77.70 ± 0.51^{b}	324.33 ± 3.09^{c}
	T4	6.03 ± 0.17^{ab}	13.99 ± 0.06^{b}	1.89 ± 0.05^{a}	212.33 ± 8.22^{b}	53.47 ± 6.25^{d}	363.00 ± 5.72^{b}
	T5	5.60 ± 0.24^{bc}	14.37 ± 0.08^{a}	1.89 ± 0.05^{a}	234.67 ± 8.99^{a}	41.10 ± 3.62^{e}	384.07 ± 5.28^{a}
	T6	5.37 ± 0.09^{c}	8.46 ± 0.11^{g}	1.25 ± 0.03^{c}	169.33 ± 4.92^{d}	36.80 ± 2.14^{e}	387.00 ± 4.97^{a}
2020	CK	5.53 ± 0.21^{abc}	10.58 ± 0.21^{e}	1.14 ± 0.16^{c}	234.67 ± 17.02^{cde}	59.98 ± 5.30^{c}	97.33 ± 18.37^{bc}
	T1	5.70 ± 0.08^{a}	16.71 ± 0.33^{b}	1.18 ± 0.14^{bc}	176.67 ± 14.29^{e}	55.53 ± 6.65^{c}	85.67 ± 11.59^{bc}
	T2	5.63 ± 0.01^{a}	12.08 ± 0.15^{d}	1.28 ± 0.12^{bc}	216.33 ± 9.43^{de}	61.65 ± 3.08^{c}	80.67 ± 5.25^{c}
	T3	5.57 ± 0.09^{ab}	15.20 ± 0.09^{c}	1.62 ± 0.17^{ab}	286.00 ± 9.09^{bc}	82.10 ± 2.83^{b}	107.33 ± 0.94^{bc}
	T4	5.20 ± 0.14^{bc}	17.29 ± 0.01^{b}	1.74 ± 0.25^{a}	366.33 ± 24.90^{a}	103.37 ± 10.05^{a}	150.00 ± 17.66^{a}
	T5	5.17 ± 0.29^{c}	18.07 ± 0.16^{a}	1.29 ± 0.30^{bc}	307.00 ± 24.26^{ab}	82.93 ± 12.00^{b}	112.33 ± 6.65^{b}
	T6	5.20 ± 0.14^{bc}	13.38 ± 0.53^{d}	1.14 ± 0.11^{c}	241.67 ± 60.50^{cd}	55.82 ± 8.92^{c}	114.00 ± 17.66^{b}

Data are expressed as mean ± S.D. ($n = 3$). Different lowercase letters indicate significant differences ($p < 0.05$).

3.2. Effect of Fertilization Management on the Quality of Tea

The quality of tea is a key factor impacting the economic value of a tea plantation. To evaluate the function of fertilization management in tea plantations, the quality of tea was analyzed under the different fertilization treatments in 2019 and 2020 (Table 2). The ratio of tea polyphenols (TPs) to amino acids (TP/AA) is an important assessing parameter for tea quality. The relatively higher amino acids content and lower polyphenols content in new shoots of spring tea, in comparison with those in the shoots of summer and autumn tea, results in lower TP/AA, which benefits tea quality. In 2019, the fertilization treatments did not significantly affect the content of caffeine, whereas the content of TPs reached a minimum in T3 (Table 2). TP/AA and the content of WEs reached a maximum in T4 (Table 2). After the second fertilizer applications in 2020, the content of FAA was significantly higher; on the other hand, the contents of TPs, caffeine, and TP/AA were significantly lower under T4 treatment than those of the other treatments (Table 2).

Table 2. Effect of different fertilization management on the quality of tea.

Year	**Sample**	**TPs (g·100 g^{-1})**	**FAA (g·100 g^{-1})**	**Caffeine (g·100 g^{-1})**	**TP/AA**	**WEs (g·100 g^{-1})**
2019	CK	15.00 ± 0.16 ^{c}	4.00 ± 0.22 ^{b}	2.80 ± 0.22 ^{a}	3.76 ± 0.22 ^{abc}	47.00 ± 0.16 ^{abc}
	T1	15.60 ± 0.22 ^{ab}	4.50 ± 0.08 ^{a}	2.80 ± 0.16 ^{a}	3.47 ± 0.10 ^{c}	46.80 ± 0.29 ^{abc}
	T2	15.10 ± 0.22 ^{bc}	4.20 ± 0.14 ^{a}	2.50 ± 0.14 ^{a}	3.60 ± 0.07 ^{bc}	47.00 ± 0.24 ^{abc}
	T3	14.70 ± 0.37 ^{c}	4.10 ± 0.16 ^{b}	2.60 ± 0.24 ^{a}	3.59 ± 0.15 ^{bc}	46.70 ± 0.16 ^{bc}
	T4	16.00 ± 0.16 ^{a}	4.10 ± 0.08 ^{b}	2.70 ± 0.22 ^{a}	3.90 ± 0.07 ^{ab}	47.50 ± 0.22 ^{a}
	T5	15.80 ± 0.24 ^{a}	4.10 ± 0.16 ^{b}	2.70 ± 0.22 ^{a}	3.86 ± 0.19 ^{ab}	46.30 ± 0.14 ^{c}
	T6	16.00 ± 0.24 ^{a}	4.00 ± 0.08 ^{b}	2.80 ± 0.14 ^{a}	4.00 ± 0.02 ^{a}	47.40 ± 0.64 ^{bc}
2020	CK	17.60 ± 0.36 ^{d}	4.50 ± 0.24 ^{ab}	4.00 ± 0.14 ^{a}	3.90 ± 0.23 ^{b}	47.30 ± 0.24 ^{e}
	T1	17.60 ± 0.22 ^{d}	4.40 ± 0.29 ^{ab}	3.70 ± 0.16 ^{ab}	4.00 ± 0.21 ^{b}	46.40 ± 0.24 ^{f}
	T2	18.70 ± 0.24 ^{b}	4.00 ± 0.22 ^{b}	3.60 ± 0.22 ^{ab}	4.70 ± 0.28 ^{a}	48.70 ± 0.16 ^{c}
	T3	17.90 ± 0.16 ^{cd}	4.40 ± 0.16 ^{ab}	3.50 ± 0.24 ^{ab}	4.10 ± 0.14 ^{b}	48.10 ± 0.16 ^{d}
	T4	16.50 ± 0.33 ^{e}	4.80 ± 0.14 ^{a}	3.40 ± 0.16 ^{b}	3.40 ± 0.17 ^{c}	48.60 ± 0.08 ^{c}
	T5	18.20 ± 0.22 ^{bc}	4.50 ± 0.22 ^{ab}	3.40 ± 0.24 ^{b}	4.00 ± 0.14 ^{b}	49.20 ± 0.24 ^{b}
	T6	19.80 ± 0.16 ^{a}	4.00 ± 0.08 ^{b}	3.70 ± 0.22 ^{ab}	5.00 ± 0.09 ^{a}	49.80 ± 0.16 ^{a}

Data are expressed as mean ± S.D. (n = 3). Different lowercase letters indicate significant differences (p < 0.05).

3.3. Effects on the Diversity of Soil Bacterial Community

We used the categorical operation on the optimized sequences to label and assess the bacterial communities in soils. A petals diagram was used to show the number of OTUs contained under the seven treatments (Figure 1). In total, in this study there were 34,387 OTUs and 51,251 OTUs in 2019 and 2020, respectively, for all samples at the 3% dissimilarity threshold. The numbers of core OTUs were 537 and 847 in 2019 and 2020, respectively. The specific numbers of OTUs in the seven treatments were analyzed by ANOVA. The number of OTUs was always greater in 2019 than it was in 2020, indicating that the use of compost over a long period of time resulted in an increase in OTUs (Figure 1).

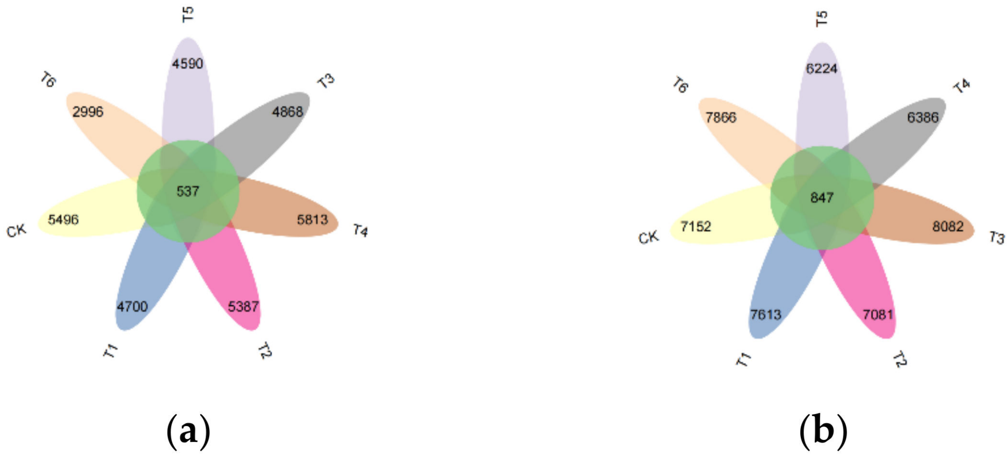


Figure 1. Petals diagram analysis of the samples in 2019 (**a**) and 2020 (**b**). Each petal represents a group, the number in the middle represents the number of OTU shared by all groups, and the number on the petal represents the number of OTU unique to the group.

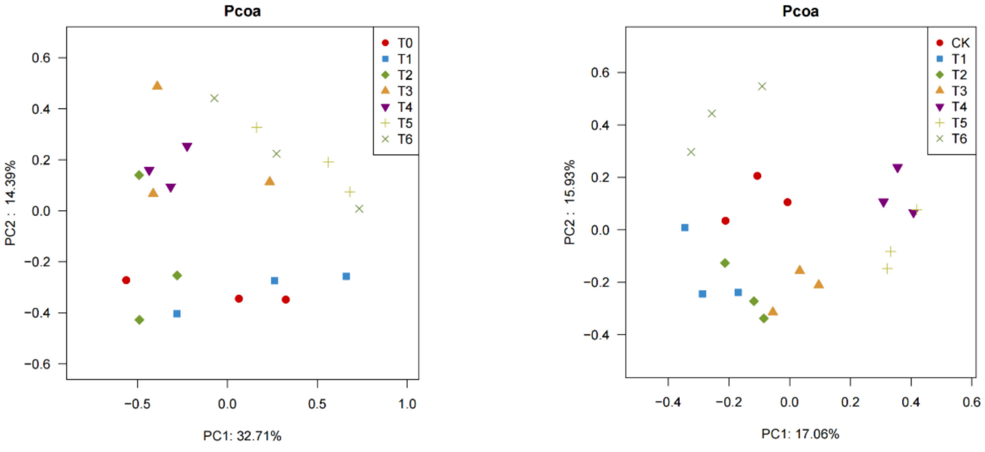
The diversity and richness of the soil's bacterial community were assessed in this research using the Chao1, Shannon, and Simpson diversity indices under the conditions of seven different treatments (Table 3). The outcomes demonstrated that various fertilizer applications had an impact on the variety of soil bacterial communities. In 2019, Shannon and Chao1 both show a consistent pattern of change. The differences in the soil bacterial community's richness index were not significant under no fertilizer, compost alone, and compost mixed with chemical fertilizers, and the lowest value was observed under the chemical fertilizer alone treatment in both Shannon and Chao1 (T6, Table 3). The highest values were observed under the compost mixed with chemical fertilizer treatment in Shannon (T4, Table 3). As a result, the variety of soil bacterial communities was low when chemical fertilizers were used alone, and the diversity of these communities was significantly impacted when the wheat straw compost was added to the chemical fertilizer.

Table 3. The diversity indices of soil bacterial community.

Year	Sample	Chao1	Shannon	Simpson
2019	CK	5175.443 ± 848.703 ^{a}	9.770 ± 0.860 ^{ab}	0.990 ± 0.010 ^{a}
	T1	4274.222 ± 784.761 ^{ab}	9.150 ± 1.040 ^{ab}	0.981 ± 0.015 ^{a}
	T2	5197.073 ± 266.914 ^{a}	10.247 ± 0.166 ^{ab}	0.997 ± 0.001 ^{a}
	T3	4891.523 ± 684.683 ^{ab}	9.969 ± 0.489 ^{ab}	0.996 ± 0.003 ^{a}
	T4	5686.680 ± 541.389 ^{a}	10.354 ± 0.244 ^{a}	0.996 ± 0.001 ^{a}
	T5	4184.350 ± 530.579 ^{ab}	8.618 ± 0.618 ^{b}	0.982 ± 0.007 ^{a}
	T6	3471.300 ± 809.504 ^{b}	8.624 ± 0.936 ^{b}	0.983 ± 0.012 ^{a}
2020	CK	5893.797 ± 329.853 ^{ab}	10.978 ± 0.136 ^{a}	0.999 ± 0.000 ^{a}
	T1	6613.487 ± 414.136 ^{a}	11.177 ± 0.157 ^{a}	0.998 ± 0.001 ^{a}
	T2	6512.830 ± 432.754 ^{a}	11.197 ± 0.115 ^{a}	0.999 ± 0.002 ^{a}
	T3	6615.643 ± 319.277 ^{a}	11.220 ± 0.034 ^{a}	0.999 ± 0.000 ^{a}
	T4	5486.453 ± 474.400 ^{b}	10.922 ± 0.234 ^{a}	0.998 ± 0.001 ^{a}
	T5	5889.917 ± 323.874 ^{ab}	10.991 ± 0.149 ^{a}	0.999 ± 0.000 ^{a}
	T6	5984.617 ± 460.159 ^{ab}	11.122 ± 0.121 ^{a}	0.999 ± 0.000 ^{a}

Data represent the average of three replicates ± standard deviations. Same lowercase letters indicate no significant differences ($p < 0.05$) among the different treatments.

The community structures of soil bacteria were evaluated using principal co-ordinates analysis (PCoA) (Figure 2). Soil bacterial communities clustered to a certain extent according to different treatments in 2019 (Figure 2a). PCoA ordination revealed distinct differences in the bacterial communities among different fertilizer treatments after two years of fertilization treatment. Soil bacterial communities strongly clustered according to different treatments in 2020; T4 and T5 in particular showed a clear separation from the other fertilization regimes (Figure 2b).



(**a**) (**b**)

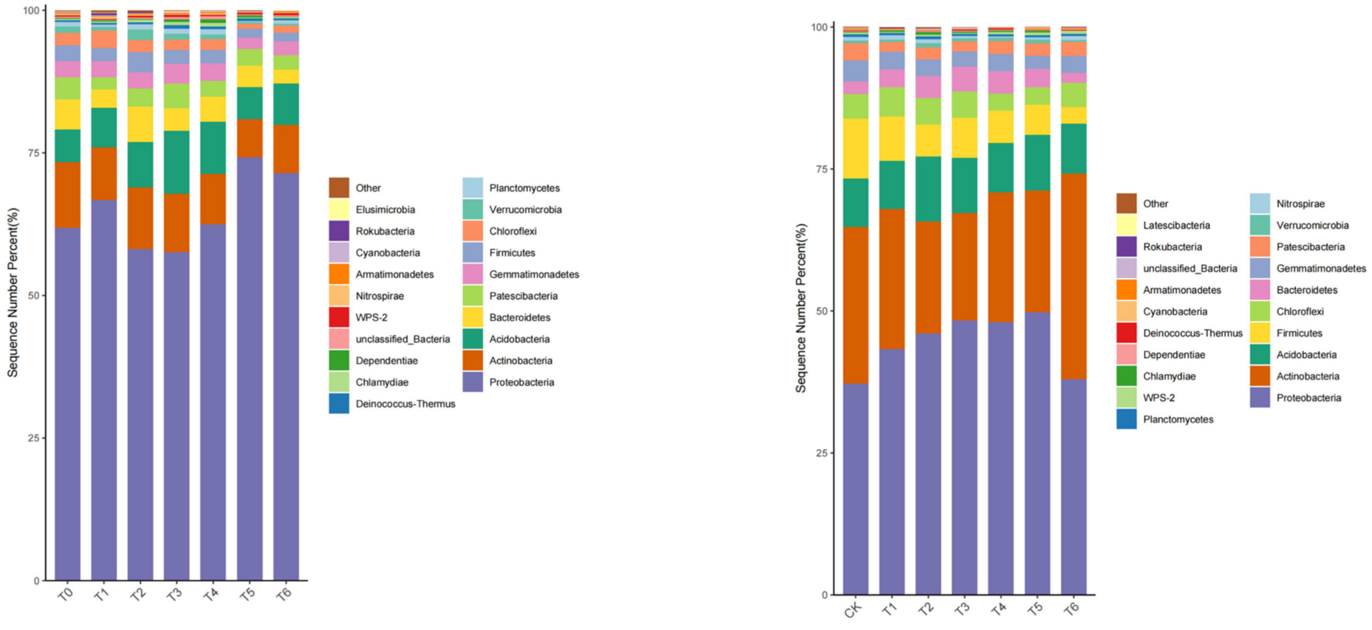
Figure 2. Principal co-ordinates analysis (PCoA) of the soil bacterial community under the different treatments in 2019 (**a**) and 2020 (**b**).

3.4. Effects on the Composition of Soil Bacterial Community

An analysis of the top 20 and 30 species with the greatest abundance at the phylum and genus levels was undertaken to determine the make-up of the soil bacterial community, respectively (Figure 3). Over the course of two years, fertilization management had a substantial impact on the makeup of the soil bacterial community. Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Patescibacteria accounted for about 80% of the relative abundance of all phyla in the bacterial population in 2019 (Figure 3a). Relative to the other treatment, T5 treatment significantly increased the relative abundance of Proteobacteria ($p < 0.05$) and decreased the relative abundance of Actinobacteria, Acidobacteria, and Chloroflexi ($p < 0.05$, Figure 3a). A species analysis conducted in 2020 revealed that Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, and Chloroflexi were the five phyla that made up the majority of the dominant bacterial communities in soils; the other phyla made up a relatively minor portion of all phyla (Figure 3b). The relative abundance of Proteobacteria ($p < 0.05$) increased and the relative abundance of Actinobacteria decreased under compost mixed with chemical fertilizer and compost alone application, compared with the unfertilized treatment (CK) and chemical fertilizer treatment (T6). On the genus level, the dominant bacteria were Acinetobacter, Rhodanobacter, Gaiellales, Micropepsaceae, and Saccharimonadales in 2019 (Figure 3c). Burkholderia and Bacillus replaced Acinetobacter and Saccharimonadales as dominant bacterial genus in 2020 (Figure 3d).

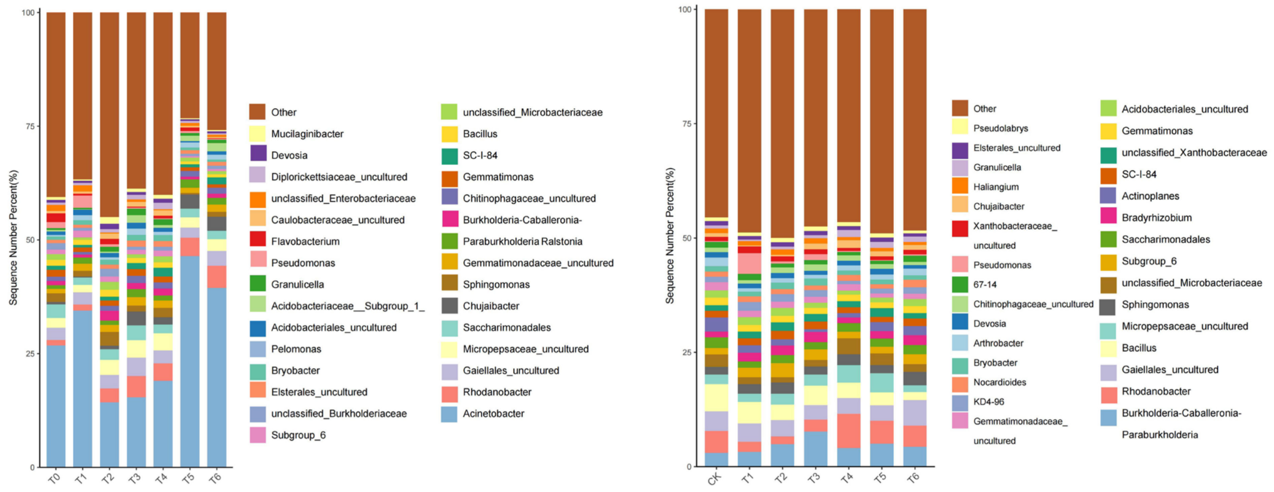
3.5. Correlation Analysis between Bacterial Communities and Soil Properties

Pearson correlation was used. The findings showed a favourable correlation between Dependentiae and SOM, TN, and AN. Chloroflexi, Latescibacteria, and Elusimicrobia were positively correlated to AP and pH, but had negative correlation with AK in 2019 (Figure 4a). SOM, TN, AN, and AP were all significantly correlated with proteobacteria. In 2020, Chloroflexi and Rokubacteria had negative correlations with TN, AN, AP, and AK but positive correlations with pH (Figure 4b).



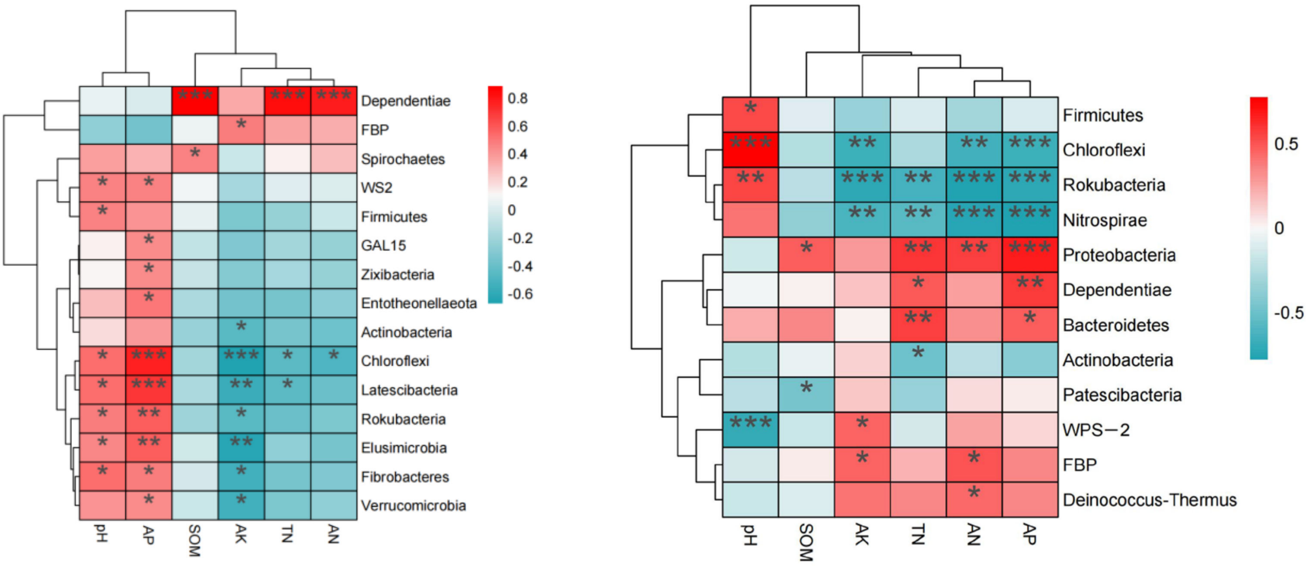
(**a**) (**b**)

Figure 3. *Cont.*



(**c**) (**d**)

Figure 3. Relative abundance of soil bacterial community under the different treatments at the phylum level in 2019 (**a**) and 2020 (**b**) and at the genus level in 2019 (**c**) and 2020 (**d**).

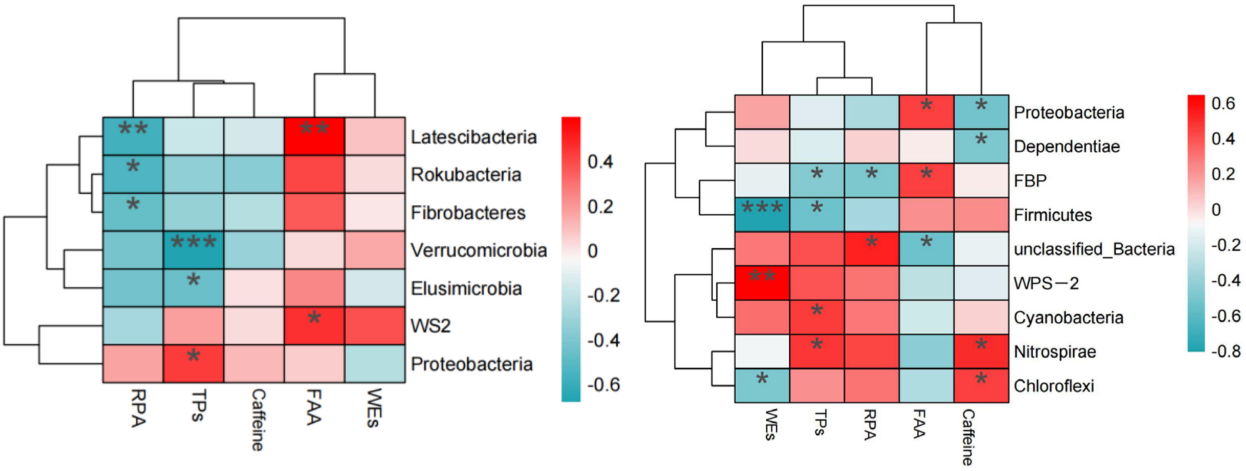


(**a**) (**b**)

Figure 4. Spearman's correlation between bacterial communities and soil properties in 2019 (**a**) and 2020 (**b**) under different fertilizer treatments. *** represents $p < 0.001$, ** represents $p < 0.01$, * represents $p < 0.05$.

3.6. Correlation Analysis between Bacterial Communities and the Quality of Tea

Correlation analysis between bacterial communities and the quality of tea showed that Proteobacteria was positively correlated with TPs and Verrucomicrobia was negatively correlated with TPs in 2019 (Figure 5a). Latescibacteria was positively correlated to FAA, but had a negative correlation with Ratio of polyphenols to amino acids (Figure 5a). In 2020, Proteobacteria was positively correlated with FAA, but negatively with Caffeine (Figure 5b).



(**a**) (**b**)

Figure 5. Spearman's correlation between bacterial communities and the quality of tea in 2019 (**a**) and 2020 (**b**) under different fertilizer treatments. *** represents $p < 0.001$, ** represents $p < 0.01$, * represents $p < 0.05$.

4. Discussion

The fertilizer contribution rate to increasing tea production far surpassed that of soil and the workforce [29]. However, long-term excessive application of chemical fertilizer causes soil acidification, resulting in a nutrient imbalance in tea gardens, which is not conducive to sustainable development. Fertilization management has been widely applied in tea plantation management [12,30–32]. Combining the use of biofertilizers is thought to be a successful method of fertilization for maintaining agricultural growth that is sustainable [33,34]. The application of organic fertilizer improved SOM as well as TN, TP, AP, and AK [35]. In the current research, fertilization management significantly changed the physiochemical properties of the soil. Wheat straw compost alone application significantly enhanced soil pH compared with chemical fertilizers alone ($p < 0.05$), either immediately or subconsciously affected soil quality and ecosystem functioning (Table 1). Soil of tea plantations prefers acidic conditions, and pH 4.5–5.5 was considered as the optimum condition. The application of a combination of compost and chemical fertilizer adjusted pH value to a suitable range (5.17–5.20) and significantly increased the content of SOM, TN, AN, AP, and AK, compared with chemical fertilizer alone and compost alone, after two consecutive years of fertilization treatments (Table 1). Therefore, it may be essential for enhancing soil efficacy in the tea plantation to neutralize the pH of the soil through the application of compost substitute.

The tea quality, as the guarantee of economic benefits, was significantly affected by fertilization managements [36]. When assessing the impact of fertilizer management on tea quality, tea polyphenols, free amino acids, and caffeine contents were typically used as assessment indicators. In this study, free amino acid content increased significantly with compost supply (Table 2), which is in line with the previous results [36,37]. Additionally, polyphenols are what give tea its sour and acerbic tastes. They also influence the tea's nutritional value. Therefore, for the preparation of green tea, a large total polyphenol content in tea leaves is not preferred. In our study, the content of TPs significantly decreased under compost treatments compared with the chemical fertilizer alone after two years application (Table 2). All indices considered, T4 treatment was found to be most optimal in this study. The quality of tea was improved by the application of compost mixed with chemical fertilizers through increasing free amino acid contents while reducing the TP/AA ratio of tea leaves.

It is believed that soil microbial diversity is essential for the safety, sustainability, and performance of soil ecosystems [24,38]. The microorganism community in the soil and its functional diversity may be significantly altered by different fertilization techniques [25,30,39]. The evaluation of soil health and the impact of chemical manure could be improved by identifying the differences in soil bacteria between various fertilized and unfertilized soil [40].

Acidobacteria, Proteobacteria, Actinobacteria, and Chloroflexi were the most abundant phyla in soils with different fertilization in tea plantation [39]. However, little is known about the variety of soil microbes in tea plantations that use only wheat straw compost and a combination of chemical fertilizers. Proteobacteria, Actinobacteria, and Acidobacteria were the three dominant (relative abundance > 10%) phyla, according to the findings of the current study, which examined the composition of the soil bacterial community at the phylum level. These findings were consistent with those of a previous study [24].

Many Proteobacteria are thought to be copiotrophic because they develop relatively quickly and utilize a variety of substrates [41]. In comparison to the unfertilized and chemical fertilizer treatments in 2020, proteobacteria revealed a greater relative abundance in compost mixed with fertilizer treatments (Figure 3b). In accordance with this research, Zhang et al., found that the abundances of Proteobacteria significantly increased in soils with cow manure fertilization but decreased with urea fertilization [27]. The abundance of Proteobacteria correlated positively with the soil properties (SOM, TN, AN, and AP, Figure 4b) and the quality of tea (TPs, Figure 5a, FAA, Figure 5b), and negatively with Caffeine (Figure 5b). The accumulation of available substrates and nutrients caused by the replacement of organic fertilizer for inorganic fertilizer may be the cause of the increases in the relative abundance of proteobacteria in the compost treatment [39]. Actinobacteria are less competitive in resource-rich environments than Proteobacteria, according to the copiotroph-oligotroph trade-off theory. Proteobacteria may be well suited to carbon-rich environments [42].

Actinobacteria are well-known to be the common inhabitants of soils that contribute to the decomposition of organic matter. In the study, the relative abundance of Actinobacteria in soils under compost mixed chemical fertilizer treatments was significantly lower than that of in soils under unfertilized and chemical fertilizer alone ($p < 0.05$), indicating that the application of compost created a relatively nutrient environment. The results were consistent with the former studies that investigated other organic fertilizer applications [12,25,31,39]. The abundance of Actinobacteria was inversely correlated with AK (Figure 4a) and TN (Figure 4b). All above results indicated that Proteobacteria and Actinobacteria could be considered as indicators to predict soil nutrition status.

In contrast to the common taxa with high stability, the soil rare taxa community was remarkably affected by environmental conditions because of their high sensitivity. The functional variety was maintained in large part thanks to rare taxa. Additionally, there was a significant positive correlation between projected functional diversity and species richness in rare taxa as opposed to common taxa [43]. In the study, changes in rare taxa community composition and diversity were observed under different fertilizer treatments during two years. According to the findings of the correlation analysis, there is a good link between rare taxa, the physicochemical makeup of the soil, and tea quality. For instance, following the first compost substitution application in 2019, Latescibacteria, Rokubacteria, Elusimicrobia, and Fibrobacteres, et al., were substantially positively associated with soil pH and AP and inversely associated with AK (Figure 4a). Verrucomicrobia and Elusimicrobia were significantly negatively correlated with TPs, whereas Latescibacteria and WS2 were substantially positively correlated with FAA (Figure 5a). Communities of rare taxa underwent a shift in structure and composition following the second compost substitution application in 2020 (Figure 4). According to the findings of the correlation analysis, Rokubacteria strongly correlated positively with soil pH and negatively with TN, AN, AP, and AK.

In summation, we concluded that regular applications of chemical fertilizer and composted wheat straw changed the makeup of the soil's microbial community, raised soil fertility, and enhanced the quality of the tea. To enhance our understanding of soil ecosystem function following straw compost application, future studies should focus on the functional diversity of bacteria.

5. Conclusions

This research focused on the effects of applying wheat straw compost mixed with chemical fertilizers for two straight years on soil bacterial diversity and community structure. Comparatively to compost alone and chemical fertilizer alone application, compost mixed with chemical fertilizers greatly increased soil bacterial diversity and successfully controlled the structure of soil bacterial communities. Moreover, bacterial communities were relevant to soil properties and tea quality. Chemical fertilizer reduction by 30%, namely, compost substitution 70% (compost fertilizers 15,000 kg ha^{-1} + chemical fertilizers 1050 kg ha^{-1}) was most effective and recommended. Therefore, it indicates that compost partly-substitution fertilization can effectively improve soil fertility and tea quality by increasing microbial diversity and provides a solution for promoting a sustainable soil ecological environment of tea plantation.

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