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Original Research

Effects of Long-Term Organic Fertilizer Application on Tea Plantation Soil of Its Physical and Chemical Properties and Microbial Communities

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Abstract

Chemical fertilizer is widely used in agricultural fertilization, but over-fertilization has caused soil quality degradation. Some studies have shown that the application of organic fertilizer is beneficial to soil quality, but there are few studies researching the long-term organic fertilizer application effect on tea plantation soil. In this paper, we studied the physical and chemical properties and microbial communities of tea plantation soil through long-term field experiments by applying organic fertilizer with equal nitrogen amounts. The experiment showed that the soil's physical and chemical properties were improved after long-term application of total organic fertilizer. Respectively, the soil organic matter (SOM), available potassium (K), available zinc (Zn), available copper (Cu), and pH were significantly enhanced, the soil bulk density was significantly increased, the water-stable aggregates were improved, the soil microbial diversity was increased. It was found that the main bacteria in tea soil were *Proteobacteria, Acidobacteria,* and *Actinobacteria*. Moreover, results from redundancy analysis showed that the SOM ($p = 0.001$), Alkali hydrolyzed nitrogen ($p = 0.049$) and pH ($p = 0.008$) had significant effects on the soil bacterial community composition. Thus SOM increased the relative richness of non-dominant bacteria, such as *Bacteroidetes, Gemmatimonadestes,* and *Firmicutes*. Overall, our results suggest that the use of organic fertilizer instead of chemical fertilizer could effectively improve the quality of tea plantation soil.

Keywords: soil properties, organic fertilizer, microbial diversity, redundancy analysis

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Introduction

Soil plays an important and fundamental role in agricultural resources and environment development, especially in water storage, nutrient cycling, and crop production. Chemical fertilizer is widely used in agricultural fertilization, but its overuse has caused soil quality degradation, like the overuse of synthetic nitrogen(N) fertilizer is often cited as a major factor in soil acidification in farmland [1, 2], but the soil pH value between 4.5~6.0 is most suitable for tea planting. Since tea plants mainly harvest shoots and young leaves, there is a high demand for fertilizer during growth [3]. It has been reported that the annual use of chemical fertilizers has exacerbated the degree of soil acidification of tea plants, seriously damaged the physical and chemical properties of the soil and the diversity of microbial communities, and led to the reduction of soil fertility. The proper use of organic fertilizer instead of chemical fertilizer can not only meet the needs of tea tree growth, but also reduce the excessive use of synthetic nitrogen(N) fertilizer and avoid the decline of soil fertility.

Organic fertilizer is mainly made from the fermentation and decomposition of agricultural and animal husbandry wastes. The organic fertilizer application can provide nutrients needed for plant growth. Studies have found those organic fertilizers made from poultry manure are rich in a variety of organic acids, peptides, and rich nutrients including nitrogen, phosphorus, and potassium, thus are able to provide micronutrients for crop growth [4], alleviate soil acidification and improve soil quality [5, 6]. Zheng et al. found that long-term application of organic fertilizer was beneficial to the improvement of soil conductivity, and significantly increased the activities of Cu, Zn, Fe, Mn, Ca, Mg, macronutrients, available nutrients, and enzymes [7]. Qaswar et al. found that the application of organic fertilizer could increase the total amount of soil nutrients such as SOM, total nitrogen (TN), and total phosphorus (TP), and also increase the content of available nutrients - Alkali hydrolyzed nitrogen (AN), available phosphorus (AP), and available potassium (AK) in soil [8]. Other studies have shown that organic fertilizers can alter the physical and chemical properties of soil, thereby reducing or eliminating the negative effects on soil quality due to chronic or excessive chemical fertilizer application [9-11]. Soil mechanical composition, bulk density, and other characteristics can affect soil moisture content, and appropriate application of organic fertilizer can reduce soil bulk density, increase soil porosity percentage, improve soil aggregates, and improve soil physical properties [12]. Sihi et al. found that the long-term application of organic fertilizers in northern India resulted in lower soil bulk density and higher water-holding capacity compared to conventional inorganic fertilization [13]. In addition, changes in soil physical and chemical properties may also affect the changes in soil microbial community structure, which in turn affect the adsorption and release of soil nutrients.

As the most active components of terrestrial ecosystems, soil microbes can regulate the functions and cycles of soil ecosystems, can play an important role in the process of element cycling, and pollutant degradation, and can sensitively reflect the changes in soil nutrients, pH, and other external conditions according to different microbial community characteristics [14, 15]. Therefore, it is of great significance to study the changes in soil microbial community after the application of organic fertilizer. Cui et al. showed that long-term application of organic fertilizer increased the abundance of *Proteobacteria*, and *Firmicutes*, *Actinobacteria,* and *Planctomycetes* were the most abundant bacteria in soils treated with combined organic fertilizer and chemical fertilizer application [16]. In addition, there are studies showed that the application of organic fertilizer affects the composition of the soil bacterial community in apple orchards - the richness of *Gammaproteobacteria, Alphaproteobacteria*, and *Rhizobiales* increases in 0-60cm soil, while the richness of *Acidobacteria* and *Sphaerobacter* decreases significantly [17]. There have been numerous studies on the changes of soil microbial communities, though there aren't studies on the changes of microbial communities in tea plantations after long-term application of organic fertilizer, so the response relationship between ecological environment changes and fertilization methods is insufficient.

Therefore, we carried out a five-year field trial on tea plantation soil to compare the application of organic fertilizers and equal-amount-nitrogen organic fertilizers. The objectives include: (1) analyzing the soil changes on the physical and chemical properties; and, (2) discussing the soil microbial community diversity after organic fertilizer application.

Materials and Methods

The Experiment Design

The experimental field, with acid yellow soil, is located in Meitan County, Zunyi City of Guizhou Province, China (107.539108 east longitude and 27.712395 north latitude). The planted tea variety in the field is Qianbei tea (number 601). The experiment was conducted in 2018, and the sample soil was collected in 2023. During the experiment, we used the same irrigation, insecticide, and weeding measures as those of conventional management measures in a 0.04 hectare experimental area. We designed 4 treatment groups, each repeating 3 times (30 m^2 per plot group), and kept tea trees in 120 cm row space and 55 cm plant space. The CK group was unfertilized, the CG group was put only chemical fertilizer, the ST group was put 60% chemical fertilizer and 40% organic fertilizer, and the YG group was put only organic fertilizer with proper ratio according to the growth of tea plants. The chemical fertilizers, with urea (N 46%), general calcium (P_2O_5 12%), and potassium sulfate (K_2O 50%) are purchased from Suzhou Yisaixin Chemical Technology Co., Ltd. The organic fertilizer is a compost of poultry manure and straw (16.8% organic matter fresh base, 1.04% nitrogen, 0.35% P_2O_5, and 0.96% K_2O). According to the actual soil conditions, we set the optimal application rates of nitrogen (N), phosphorus (P), and potassium (K), respectively, 300 kg•ha^{-1}, 35.1 kg•ha$^{-1,}$ and 83.4 kg•ha^{-1}. Ensuring consistent nitrogen application rate in each treatment group, and set the fertilization rates as follows: CG group (urea 652.2 kg•ha-1, calcium 837.45 kg•ha-1, potassium sulfate 201 kg•ha^{-1}), ST group (urea 391.05 kg•ha^{-1}, calcium 502.5 kg•ha^{-1}, potassium sulfate 120.6 kg•ha^{-1}, organic fertilizer 11538.45 kg•ha^{-1}), YG group (organic fertilizer 28845.75 kg•ha^{-1}).

The Sample Soil Collection

A total of 12 samples of fresh topsoil (0-20 cm) around the roots of tea plants were collected by the five-point sampling method for each treatment group. Each soil sample was about 500 g, packed in plastic bags, and 120 g of it was refrigerated while the remaining soil was air-dried indoors after removing residues and debris for later experimental tests.

The DNA Extraction and High-Throughput Sequencing

Weighed 2 g refrigerated soil and sent it to Shenggong Bioengineering (Shanghai) biological testing company in time. DNA extracted using E.Z.N.A™ Mag-Bind Soil DNA Kit. Quality was evaluated by gel electrophoresis and UV spectrophotometry. The bacterial 16S rDNA V3-V4 region was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′), which contained adapter sequences and bar code labels. PCR mixture: Hieff® Robust PCR Master Mix, primers, DNA, and water. PCR program included denaturation, annealing, and extension cycles. PCR products were analyzed by gel electrophoresis and purified with a Gel Extraction Kit. DNA concentration was measured using a Qubit 3.0 Fluorometer. Procedures conducted by Shenggong Bioengineering (Shanghai) Co., Ltd.

Sample Determination

We analyzed the physical and chemical properties of 12 soil samples. First, we adopted the "volumetric cylinder method" to estimate the soil bulk density, which requires a solid ring or volumetric cylinder to be hammered or pressed into the soil to take a natural-state soil sample. Soil samples were dried and the mass of the dry soil sample was measured and estimated.

The Soil water stable aggregates were determined according to the wet sieving method described by Cambardella et al. Weighed a 100 g mixed and air-dried soil sample out and spread it evenly on a stack of sieves (0.25 mm, 0.5 mm, 1mm, 2 mm, 5 mm) and soaked them in water. Then the sieves were filled with water to ensure that the aggregates in the upper sieves were submerged at the highest point of oscillation. After a full soaking, we switched on the soil aggregate analyzer (TTF-100 from Nanjing soil) and vibrated 30 cycles min^{-1} for 20 minutes at a frequency of 4cm. After that, the soil aggregates contained in each sieve were carefully washed into a beaker, oven-dried for 12 hours at 60ºC, and then weighed to determine the mass of stable aggregates.

The pH value test was tested according to the "Determination of Soil pH" (NYT1377-2007). The specific steps: (1) Air-dry the samples in a cool and ventilated place, then weigh 10.0 g±0.1 g, pass it through a 2 mm standard test sieve, place it in a 50 ml beaker, and add water according to the ratio of 2.5: 1. The container was sealed and shaken at 25ºC with a water bath shaker for 30 minutes, and then tested with a pH meter after standing for about 2 hours.

The determination of the organic matter in soil samples adopted outside the heating method, that is, firstly weighed 0.05 g sample to pass through a 150 mm sieve, then added 5 ml prepared potassium dichromate solution and 5 ml concentrated sulfuric acid. Heated it to boil for 5 minutes in the cooking furnace, and then titrated with the configured ferrous sulfate solution. Next, the weighed 0.1 g soil sample was put through a 150 mm sieve and digested by aqua regia. At last, determined the concentration of trace elements (Fe, Mn, Cu, Zn) in the digest by flame atomic absorption spectrometer (Thermo Fisher ICE3500).

Alkali hydrolyzed nitrogen (AN) was determined alkali hydrolysis diffusion method with AA3 continuous flow analyzer (AA3, Bran + Luebbe, Hamburg, Germany). Available P (AP) was extracted with 0.5 mol/L Na bicarbonate and also quantified by the molybdenum antimony colorimetric method. For the determination of effective potassium, the sample soil was weighed through a 2 mm pore size sieve, and 5 g soil was placed in a jar to be mixed with 50 ml ammonium acetate solution. After shaking, it was filtered through a membrane and stored to preserve with the flame photometry (FP6440 flame photometer of Griver Industrial Equipment (Suzhou) Co., Ltd.). To determine the availability of trace elements like Fe, Mn, Cu, and Zn in the soil, 20ml of Morgan solution was added to the 4 g soil sample and poured into a 100 ml Erlenmeyer flask, then placed on a shaker to shake at 150 rpm for 15 minutes and filtered through filter paper to produce a clear solution. Trace element extracts (Fe, Mn, Cu, Zn) were determined by ICP-MS (NexION 300X, Perkin Elmer, Norwalk, CT, USA). Total soil nitrogen was determined by using the Jerdal method and determined by Continuous Flow Analyzer (AA3, Bran + Luebbe, Hamburg, Germany). We named two plot soil samples as TP and TK, dried them at 105ºC in an oven for 6 hours, passed them through a 0.15 mm nylon sieve, and then digested them with nitric acid and perchloric acid (4:1, v/v) by inductively coupled plasma optical emission spectrometry (ICP-OES; 710 series, Agilent Technologies, Palo Alto, CA, USA).

Statistical Analysis of Data

Prism software and Excel were used to calculate the soil's physical and chemical properties and soil microbial diversity index and to analyze the differences between groups. Redundancy analysis (RDA) was used to analyze the effects of soil chemical properties on soil microbial community composition. The microbial OTU data were analyzed by the main component analysis method (PCA) and performed by the R language software Rstudio-4.3.0. The content of water-stable aggregates (>0.25 mm) $R_{0.25}$ is calculated using equation (1) as below. The "W_i" refers to the content of aggregates in the i^{th} group (Table S1). The average mass diameter (MWD) of soil water-stable aggregates was calculated by equation (2) as below. The "X_i" refers to the average pore size of the i^{th} pore size sieve and the "i+1"st pore size sieve.

$$R_{0.3} = \sum_{i=1}^{n} W_i \quad (1)$$

$$MWD = \sum_{i=1}^{n} X_i W_i \quad (2)$$

Result

Changes in the Soil's Physical and Chemical Properties

From Table 1, the pH values of the ST group and YG group were 5.23 and 5.59, respectively, which were significantly higher than those of the CG group and the CK group. The SOM of the ST group and YG group were 53.92 g/kg and 55.58 g/kg, respectively, which were significantly increased by 1.90 times and 1.99 times compared with the CK group, which only rose 0.82 times and 0.87 times, respectively, compared with the CG group. The TN levels of the ST group and YG group were 2.15 g/kg and 2.01 g/kg, respectively, which were 72% and 60.8% higher than those in the CK group, and 32.72% and 24.07% higher than those in the CG group. The content of TP and TK has no significant differences among the ST, YG, and CG treatment groups, but significantly increased than the CK group. From the perspective of available effective states of elements, the AN has no significant differences among the three treatment groups, but significantly increased than the CK group too. In which, the highest content was 150.92 mg/kg in the YG group and the lowest content was 136.71 mg/kg in the ST group. The available potassium in the YG group, with a content of 280.95 mg/kg, was significantly up 129.67%, 43.58%, and 38.63% than that in the CK group, CG group, and ST group. The highest AP content in the CG group was 186.90 mg/kg, which was 29.07 times, 1.41 times, and 2.23 times that of the CK group, ST group, and YG group, respectively. From the comparison among soil with equal-amount-nitrogen organic fertilizer, the unfertilized soil, and the soil with conventional chemical fertilizer, we found the pH, SOM, and available potassium in the soil were significantly improved after the application of equal-amount-nitrogen organic fertilizer. Comprehensively comparing the soil nutrient content, this study considered that the soil nutrients in the YG group were more abundant and suitable for plant growth.

The mechanical composition of soil is mainly divided into sand, silt, and clay particles, which affect the soil porosity, the dissolution of organic matter, and the retention rate of water. The particle quantity in four treatment groups showed a trend of clay>silt>sand particles. The highest clay particle percentage was 63.40% of the CK group, and the lowest was 45.07% of the ST group. The highest silt particle percentage was 30.80% of the ST group and the lowest was 22.70% of the CK group. The highest sand particle

Table 1. Soil chemistry properties under different fertilization.

	CK	CG	ST	YG
pH	4.43±0.09 a	4.39±0.08 a	5.23±0.25 b	5.59±0.27 b
SOM(g/kg)	18.58±2.39 a	29.66±2.10 b	53.92±1.66 c	55.58±3.17 c
TN(g/kg)	1.25±0.08 a	1.62±0.06 ab	2.15±0.2 b	2.01±0.47 b
TP(g/kg)	0.47±0.06 a	1.31±0.26 b	1.11±0.23 b	0.92±0.18 b
TK(g/kg)	11.55±0.69 a	14.51±1.38 b	15.3±1.08 b	14.75±0.74 b
AN(mg/kg)	81.34±10.32 a	150.43±8.09 b	136.71±23.66 b	150.92±32.02 b
AK(mg/kg)	122.33±10.50 a	195.67±43.31 b	202.66±11.01 b	280.95±13.06 c
AP(mg/kg)	6.43±1.48 a	186.90±42.12 b	132.89±36.87 c	83.70±6.59 c

percentage was 23.77% of the CG group and 13.90% of the CK group.

From the perspective of soil bulk density, the soil bulk density of the CG group was the highest (1.38 g/cm^3), which was a little different from the 1 .37 g/cm^3 of the CK group. However, the soil bulk density of the ST group and YG group was significantly reduced to 87.59% and 84.67% of that in the CK group.

Water-stable macro-aggregates of over 0.25mm particle size ($R_{0.25}$) are considered to be the best structure in soil structure and play a key role in maintaining soil structure stability. The $R_{0.25}$ of CG, ST, and YG groups in fertilization treatment was 70.15~74.28, which was significantly higher than that of the CK group, and the $R_{0.25}$ value of the YG group was the largest. MWD (mean weight diameter) is an important indicator of aggregate stability. The higher the value of MWD predicates the better the stability of soil aggregates, and the higher the degree of agglomeration. It can be found that the MWD of CG, ST, and YG groups with fertilization treatment has a significant increase compared with 0.82 of CK. The highest MWD is 2.57 in the YG group, and the lowest one is 2.08 in the CG group.

From the perspective of soil physical properties, this study found that the application of organic fertilizer with an equal amount of nitrogen, compared with the unfertilized soil and the soil with conventional chemical fertilizer, promoted the formation of water-stable macro-aggregates and improved the structure of soil aggregates, among which the stability of soil aggregates in the YG group was the comprehensive optimal choice.

Effects on Tea Plantation Soil Trace Elements

In this study, it was found that the trace elements (iron, manganese, copper, zinc) in the four treatment groups were 64612.10~81510.07 mg/kg, 270.67~325.57 mg/kg, 47.29~61.06 mg/kg, and 146.13~180.43 mg/kg (Fig. 1a).

The available effective states of elements refer to the main forms that can be directly used by plants. Among the four treatment groups, the highest available iron content was 74.44 mg/kg in the CG group, and the CK group, ST group, and YG group decreased significantly by 2.43 times, 0.87 times, and 0.53 times compared with the CG group, respectively. The highest available manganese content was 16.31 mg/kg in the CK group, and the CG group, ST group, and YG group decreased significantly by 0.93 times, 1.51 times, and 2.79 times compared with the CG group, respectively. The highest available copper content was 1.56 mg/kg in the YG group, and the CK group, CG group, and ST group decreased significantly by 3.35 times, 2.04 times, and 1.21 times compared with the YG group, respectively. The highest available zinc content was 2.45 mg/kg in the YG group, which was significantly decreased by 3.15 times, 0.91 times, and 0.37 times in the CK group, CG group, and ST group compared with the YG group, respectively.

In this study, it was found that after the application of equal-amount-nitrogen organic fertilizer, compared with the soil without fertilization and the soil with conventional chemical fertilizer, the contents of available copper and available zinc in the YG group were significantly improved, while the contents of available iron and available manganese showed a decreasing trend. This may be chalked up to the fertilization of organic fertilizer changing the soil redox conditions and the structure of SOM.

Changes in Soil Microbial Diversity

The Shannon index is one of the indices used to estimate the microbial diversity in the sample. Together with the Simpson Diversity Index, it is often used to reflect the alpha diversity index. The smaller the Simpson index value is, the higher the community diversity will be. The larger the Shannon value is, the higher the community diversity will be. The Simpson indices of CK, CG, ST, and YG groups in the experimental group were 0.0174, 0.0153, 0.0118, and 0.006, respectively. (see Fig. 2), and the Simpson indices of the YG and ST groups decreased by 60.78% and 22.87%, respectively compared with the CG group. The Shannon indices of CK, CG, ST, and YG was 4.97, 5.17, 5.55, and 6.11, respectively, and the Shannon indices of YG and ST increased by 18.18% and 7.35% compared with the CG

Table 2. Soil physics properties under different fertilization.

	CK	CG	ST	YG
Sand (2-0.02mm)%	13.90±2.30a	23.77±3.12b	24.13±4.01b	18.87±2.32ab
Silt (0.02-0.002mm)%	22.70±1.31a	28.90±1.35b	30.80±4.33b	28.93±1.67b
Clay Particles (<0.002mm)%	63.40±1.22a	47.33±3.30b	45.07±7.76b	52.2±0.72b
Soil Bulk Density	1.37±0.03a	1.38±0.03a	1.20±0.08b	1.16±0.02b
$R_{0.25}$%	41.06±0.94a	70.15±4.16b	72.29±2.99b	74.28±4.67b
MWD	0.82±0.02a	2.08±0.22b	2.43±0.17c	2.57±0.19c

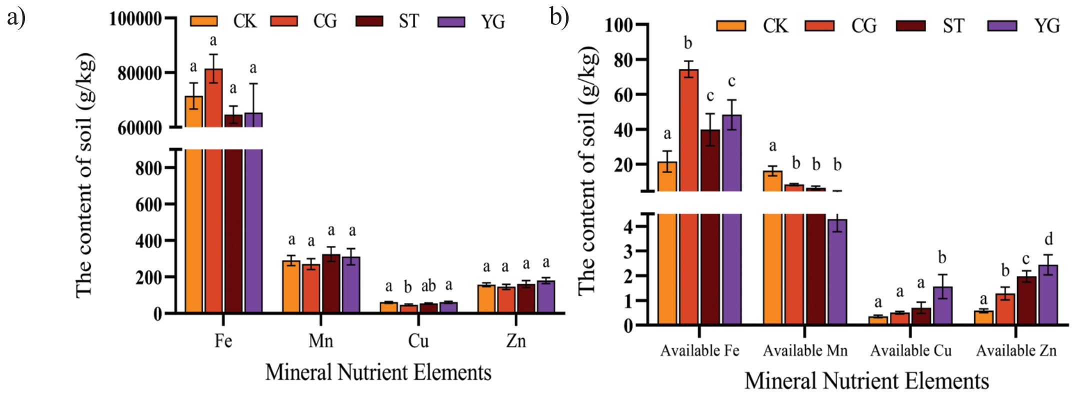

Fig. 1. Physical and chemical properties of tea plantation soil.
a) is the content of iron, manganese, copper, and zinc in soil; b) shows the available effective states of Fe, Mn, Cu, and Zn in tea plantation soils. The letters a, b, and c indicate significant differences between groups, p<0.05.

group. The results showed that the application of organic fertilizer could improve the soil microbial diversity of tea plantations, the Simpson index of the YG group was the smallest and the Shannon index was the largest among the three groups of fertilization measures.

The analysis of principal components (PC1 and PC2) for the operational taxonomic units of soil micro-organisms showed that the two principal components explained about 58.74% of the total variation, respectively, PC1 38.76% and PC2 19.98%. (see Fig. 3), these results imply that different fertilization methods have a key impact on the structure and diversity of soil bacteria in tea plantations.

We further analyzed the composition of bacterial communities in different treatment groups. At the bacterial phylum level (Fig. 4a), it was mainly distributed in 13 bacterial phyla in addition to the unclassified bacteria (relative abundance 4.74%~14.58%) and other (relative abundance 0.49%~1.32%) groups. We defined the top three phyla in bacteria as the dominant phyla, respectively, *Proteobacteria, Acidobacteria,* and *Actinobacteria*. The result showed that:

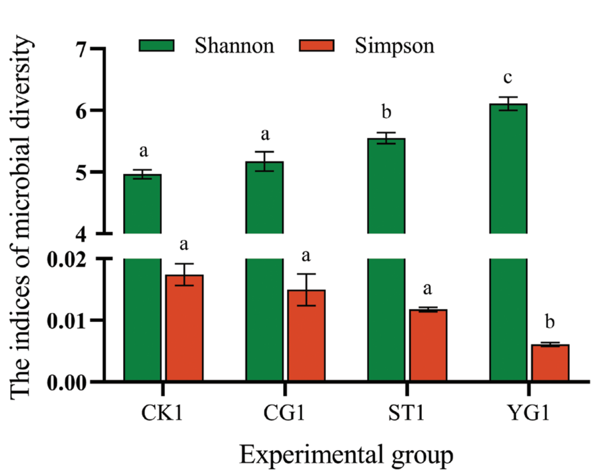

Fig. 2. Soil microbial diversity index.

(1) In the CK group, their average relative richness was 28.31%, 33.29%, and 8.91%, respectively.

(2) Compared with the CK group, the relative abundance of *Proteobacteria* in CG, ST, and YG increased, while the *Acidobacteria* decreased.

(3) Compared with the CK group, the relative abundance of *Actinobacteria* in CG increased, while it decreased in ST and YG.

(4) Among them, the relative abundance of *Proteobacteria* and *Acidobacteria* in YG and ST increased significantly compared with CG.

(5) Compared with CK and CG, the contents of non-dominant phyla *Bacteroidetes*, *Gemmatimonadestes,* and *Firmicutes* increased in ST and YG.

At the bacterial order level (Fig. 4b), the dominant bacteria in the soil were *norank_Acidobacteria_Gp1*, *Rhizobiales*, and *norank_Acidobacteria_Gp2,* and their proportions in the four experimental groups were 6.76%-14.20%, 4.23%-11.25%, and 2.61%-12.20%, respectively. The *norank_Acidobacteria_Gp1* and *norank_Acidobacteria_Gp2* in CG, ST, and YG decreased exponentially compared with CK, while the *Rhizobiales* increased exponentially. Except for the dominant bacteria, the abundance of other bacterial groups in ST and YG were 5.89% and 8.92%, which were higher than those of CK and CG; while in the CG group was 4.28%, which was lower than that of 5.68% in CK group. Therefore, the above bacterial community analysis showed that different fertilization methods would affect the richness of its bacterial community, and the soil microbial community structure was different under different fertilization methods too.

The Effect of Soil Chemistry on the Bacterial Microbial Community

The results of RDA analysis showed that the first two spindles account for 51.96% and 20.24%, respectively. When the angle between the environmental variable and the species variable was an acute angle, it indicated a positive impact on the soil bacterial community

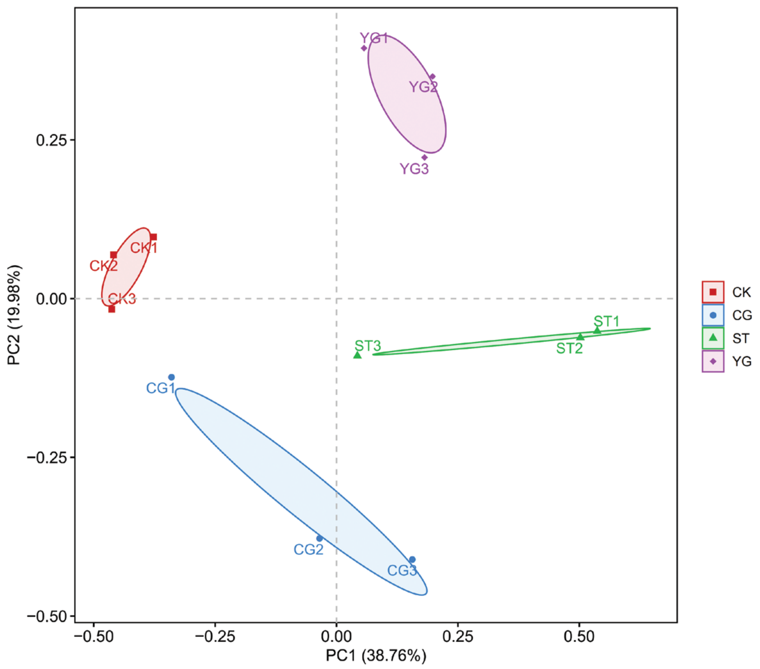

Fig. 3. PCA results of soil bacteria in different fertilization experimental groups.
(CK is fertilizer-free; CG is a pure fertilizer; ST is 60% chemical fertilizer + 40% organic fertilizer; YG is pure organic fertilizer).

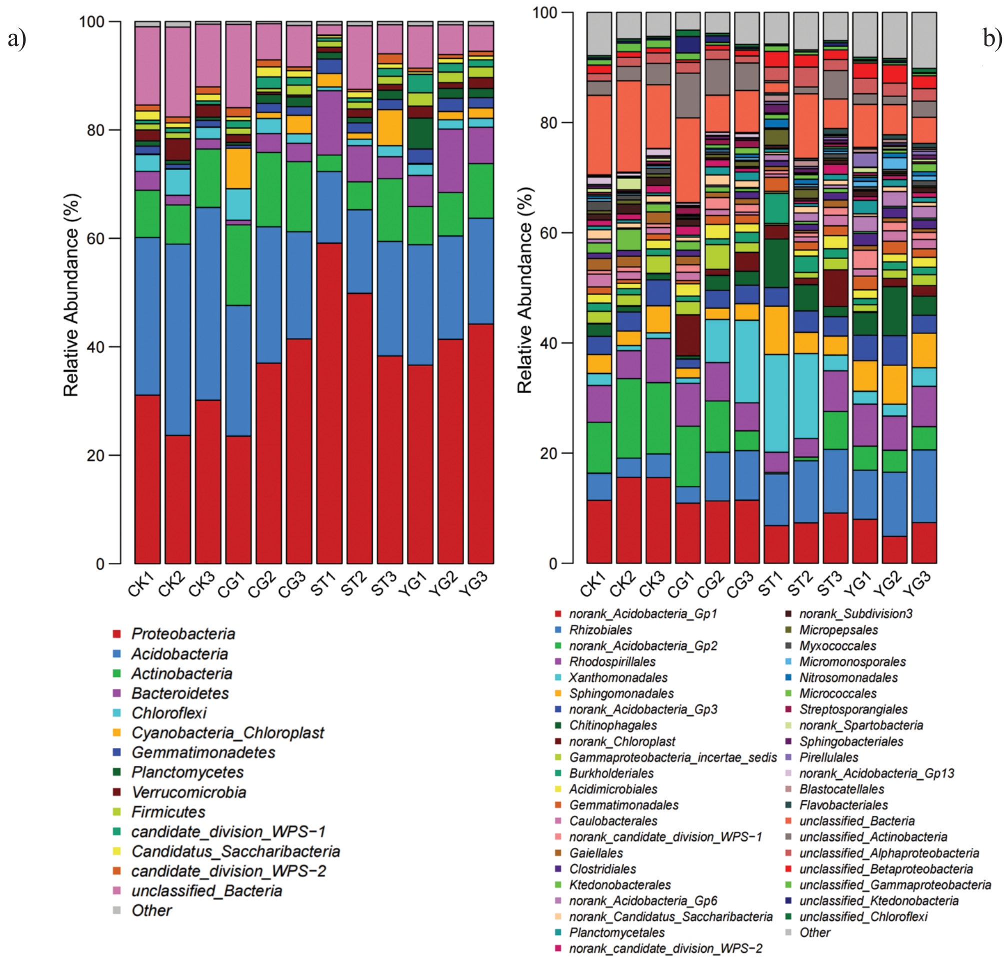

Fig. 4. Relative richness of soil bacterial communities in different treatment groups.
a) shows the relative abundance of microbial phylum levels; b) is the relative abundance of microbial bacteria levels.

Table 3. Explanatory power results of soil environmental factors.

Item	SOM	AN	TN	pH	TK	AK	AP	TP
Explains%	24.67	16.52	15.65	11.75	10.45	8.35	7.12	5.48
F	19.27	5.69	1.79	11.93	0.66	3.30	2.34	0.94
P	0.001	0.049	0.237	0.008	0.515	0.106	0.174	0.431

structure. The smaller the acute angle, the greater the positive impact, and vice versa (Fig. 5). Among them, SOM (p = 0.001), AN (p = 0.049), and pH (p = 0.008) had significant positive correlations on soil bacterial community composition. The explanation rate of SOM, AN, and pH on the bacterial community structure of soil was 52.94%, while the SOM counted the highest at 24.67%." to " The explanation rate of SOM, AN, and pH on the bacterial community structure of soil was 52.94%, while the SOM counted the highest at 24.67% (Table 3).

The SOM in soil showed a positive correlation with non-dominant phyla *Bacteroidetes, Gemmatimonadestes, Planctomycetes, Firmicutes, Candidate_division_WPS.1,* and *Cyanobacteria_Chloroplast.*

The AN in soil showed a positive correlation with non-dominant phyla *Chloroflexi, candidate_division_WPS.2, Cyanobacteria_Chloroplast, Candidate_division_WPS.1, Firmicutes, Planctomycetes,* and *Gemmatimonadestes.*

The pH in soil showed a positive correlation with non-dominant phyla *Cyanobacteria_Chloroplast, Candidate_division_WPS.1, Firmicutes, Planctomycetes, Gemmatimonadestes,* and *Bacteroidetes.*

Discussion

Organic Fertilizer Can Improve the Physical and Chemical Properties of Soil

The research showed that soil bulk density and MWD decreased significantly, while the $R_{0.25}$ value increased gradually (Table 2) after 5 years of continuous application of organic fertilizer, through which, the low decomposition rate of fertilizer could lead to more macro molecular organic matters and clay minerals exist in soil, thus favorable to the increase of the soil aggregates and the reduction of soil bulk density [18, 19]. The experiment illustrated that the organic fertilizer application can increase the soil porosity, improve aggregate stability, and enhance the soil structure.

In terms of the chemical properties of soil, we found that the application of organic fertilizers could help to increase the content of SOM, pH, and AK in soil (Table 1). Organic fertilizer contains a large amount of organic matter, and its long-term application can provide exogenous SOM input for pure soil. Shi et al. found that the pH and AK content in soil could increase significantly after applying equal-amount-nitrogen organic fertilizer with different proportions in citrus orchard soil [20-22], which is also consistent with our

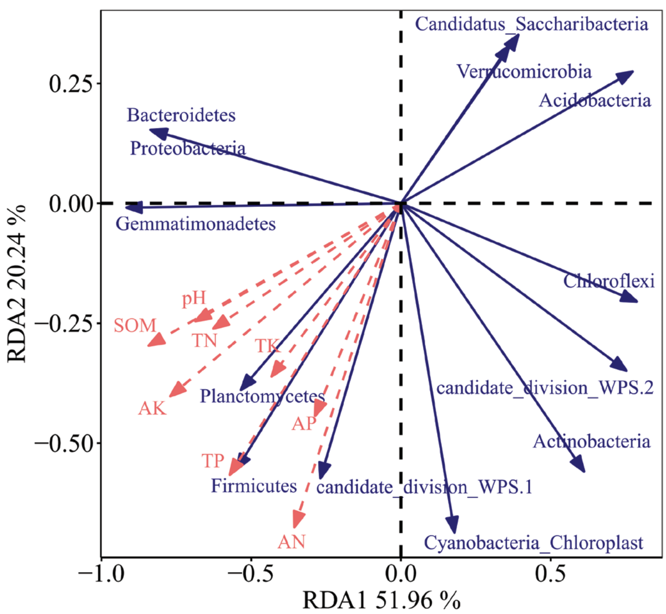

Fig. 5. Redundancy analysis of the bacterial community and soil environmental factors.

results. This may be due to the abundant K element in the organic fertilizer we applied, or due to the tea plant root exudation, which dissolved mineral nutrients in the soil [23, 24]. When the content of SOM percentage content was over 8%, the water retention capacity was mainly affected by it [25]. In our study, the SOM percentage content was over 20% and the water retention capacity improved with few-years organic fertilization [21, 26]. The application of organic fertilization is also conducive to the improvement of available K content and trace elements quantity. For example, the input of alkaline substances in organic fertilizer and the salt-based ions released by organic fertilizer during the mineralization process, can effectively neutralize soil acidity and alleviate soil acidification [27]. This is consistent with previous findings by Cai et al. that the use of organic fertilizers can alleviate soil acidification [22, 28].

The content of available trace elements is the key to soil suitability in tea plantations, and SOM also plays an important role in the accumulation, mobilization, and transportation of trace elements in the soil. The SOM interacts similarly with the elements Mn and Fe. For example, the bond strength between the Mn element and organic compound is weak, and the correlated complexation reaction between available Mn and organic matter is weak too [29, 30]. In contrast to Fe and Mn elements, copper (Cu) interacts more strongly with SOM, and it binds more to certain organic ligands than other metal trace elements, and it is more inclined to be adsorbed into organic compounds to form inner organic complexes [31]. Also, previous studies have shown that the higher the SOM content in the soil, the higher the Cu adsorption capacity [32].

In this study, it was found that there was no significant change in the total amount of trace elements applied to organic fertilizer. The content of available iron and available manganese decreased, and the content of available copper and available zinc increased. The results showed that the soil of the tea plantation was generally rich in Fe and Mn elements. Compared with Cu and Zn elements, Fe and Mn elements were easier to undergo reduction reaction to form manganese oxide and iron oxide precipitates, which made the available state of Zn^{2+} and effective Cu^{+} in the soil be released. In addition, SOM can adsorb trace elements through the organic functional groups on its surface, and trace elements can also form organic complexes with soluble SOM. These interactions are related to the specific group properties of SOM. Therefore, the application of organic fertilizer increased the SOM content and potentially decreased the content of available state iron and manganese, which is conducive to the solubility of Cu and Zn.

Effect of Long-Term Application of Organic Fertilizer on Bacterial Community

Microbial activities can affect the circulation of soil mineral elements, and various mineral elements can offer energy for microbial activities. In this study, the microbial diversity in the organic fertilizer treatment group was higher than that in the pure chemical fertilizer treatment group. The significant change in the Simpson index and Shannon index shows the enhancement of bacterial diversity. There were studies proving that organic fertilizer can provide nutrients for soil microorganisms and improve the biological activity of microorganisms [33]. Through the RDA analysis on soil chemical properties and soil bacterial community (phylum level), the important factors of bacterial structure are SOM, pH, and AN, and through correlation analysis (Fig. S1), SOM and pH are significantly positively correlated with the non-dominant phylum *Bacteroidetes, Gemmatimonadestes*, and *Firmicutes*. There were also studies have shown that the microbial biomass level of soil with long-term or short-term chemical fertilizer application is lower than that with organic fertilizer application, for elements like carbon and nitrogen were provided during the decomposition of rich SOM, and they can provide sufficient material and energy sources for microbial activities. At the same time, it also has a certain impact on microbial diversity in maintaining soil moisture and improving soil structure [34]. Our experimental results confirm it as well. Wang et al. found that the soil with organic fertilizer treatment was the most sparse, indicating that there were fewer interactions between soil microorganisms after organic fertilizer was applied [35], and a large number of soluble SOM increased soil porosity and became a new site for microbial activity, which may also be one of the reasons for the large impact of SOM on microbial diversity. The increase in soil pH was also the main reason for the increase in bacterial diversity and richness [36], and Li et al. also found that soil pH was the main factor affecting the structure of soil bacterial community after the application of organic fertilizer [37], and our results also showed that the soil pH also increased with the increase of organic fertilizer application.

Many organic colloids contained in organic fertilizer provide a material basis for the formation of organic and inorganic composite aggregates in soil, which is conducive to the production of active calcium ions and the increase of humus content. Organic fertilizers are also rich in beneficial functional groups, adding new sources of carbon and energy to the soil and promoting the formation of soluble carbon and hydrogen, thereby increasing their available energy [38]. For example, at the phylum level, *Bacteroides* in soil usually decompose organic matter, including plant residues and other organic wastes, and can also participate in the decomposition and recycling of organic matter. *Gemmatimonadestes* may be involved in the carbon and nitrogen cycling in soil, while the increase of organic matter may affect the ratio of carbon and nitrogen. *Firmicutes* participate in decomposition in the soil, decomposing organic matter. Therefore, it has also been proved that the increase of SOM can improve their biological activity, promote their metabolism and reproduction, and thus increase their abundance. In general, long-term application of organic fertilizer can improve the physical and chemical properties of soil, alleviate soil acidification, and change the soil environment, which in turn affects the soil microbial diversity and changes the soil bacterial community structure.

Conclusion

In this study, it was proved that long-term application of organic fertilizer with equal nitrogen amount can improve the soil quality of tea plantation, in which the pH value, the content of SOM, and available K were significantly increased, the content of trace elements available Zn and Cu was also significantly improved, the soil bulk density was significantly reduced, the stability of aggregates was improved, and the diversity of soil microbial community was also significantly increased. Combined with the changes in the physical-chemical properties and the microbial community structure, the full application of equal-amount nitrogen fertilizer instead of chemical fertilizer had the best effect on improving soil physical-chemical properties in tea plantations. In addition, the composition of the soil bacterial community was significantly correlated with the content of SOM, AN, and pH in the soil, which confirmed that the application of organic fertilizer instead of chemical fertilizer had an important effect on the composition and diversity of the soil microbial community structure. In a word, our study further confirms that the application of organic fertilizer can not only improve the soil physical and chemical properties of tea plantations, but also improve the diversity of soil microbial communities in tea gardens, which is conducive to the sustainable planting of tea gardens.

Authors Contributions

Jian Yang: Conceptualization, Methodology, Formal analysis, Data Curation, Visualization, Software, Writing - Original Draft.

Fang Liu: Validation, Data Curation, Writing - Review & Editing.

Jian Zhu: Sampling, Supervision, Project administration, Resources.

Zuyong Che: Supervision, Project administration.

Jie Dai: Investigation, Methodology, Project administration, Writing - Review & Editing.

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Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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