Short-Term Effects of Bio-Organic Fertilizer on Soil Fertility and Bacterial Community Composition in Tea Plantation Soils

Zhenmin Hu, Lingfei Ji, Qing Wan, Huan Li, Ronglin Li and Yiyang Yang

1 Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, Institute of Leisure Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
2 Key Laboratory of Biology, Genetics and Breeding of Special Economic Animals and Plants, Ministry of Agriculture and Rural Affairs, Hangzhou 310008, China
* Correspondence: yangyiyang_yyy@126.com; Tel.: +86-025-8439-1693
† These authors contributed equally to this work.

Abstract: Overuse of chemical fertilizers to maintain tea production has caused many adverse effects in tea plantations and largely hampers the sustainable development of the tea industry. Applying bio-organic fertilizer (BOF) to achieve the goal of sustainable agriculture has become popular because of its advantages, such as its pollution-free nature, considerable amount of beneficial microbes and soil-friendly organic materials. However, the effects of BOF application on tea plantation soil remain an open question. Herein, we carried out a 3-year pot experiment with four treatments, including control without fertilization (CK), 100% chemical fertilizer (CF), 50% chemical fertilizer +50% BOF (CFOF) and 100% BOF (OF), to explore the effects of BOF application on soil fertility and bacterial community in tea plantations. The results showed that BOF application could increase soil fertility in both bulk and rhizosphere soils and improve the biomass of tea leaves. In addition, the nutrient level change caused by BOF application significantly changed bacterial community diversity and composition and accounted for 74.91% of the community variation. CFOF and OF treatments significantly increased the bacterial Chao1 and Shannon indices compared to CF treatment (p < 0.05). Moreover, bacterial community composition was dominated by Betaproteobacteria (46.88%), Acidobacteria (11.29%), Alphaproteobacteria (9.69%) and Gammaproteobacteria (9.59%). BOF application increased the relative abundance of Alphaproteobacteria, Acidobacteria, Deltaproteobacteria and planctomycetes and decreased the relative abundance of Betaproteobacteria (p < 0.05). Furthermore, bacterial function prediction revealed that BOF application improved the N and C cycling processes and enhanced the co-occurrence network complexity in the bulk soils. Bacterial community functions and co-occurrence networks in the rhizosphere did not show similar results, indicating that rhizosphere bacterial communities were more affected by the rhizosphere effect than BOF application. All these findings verified our hypothesis that applying BOF in tea plantations could increase the biomass of tea plants by improving soil fertility and influencing the soil bacterial function groups. In summary, we suggested that BOF application could be a promising way to achieve the sustainable development of the tea industry.

Keywords: bio-organic fertilizer; bacterial community; bulk soils; rhizosphere; tea plantation soils

1. Introduction

Tea plants are one of the most popular beverage crops worldwide. Large quantities of synthetic fertilizers (e.g., urea, potassium sulphate and superphosphate) are applied in tea plantations to achieve economic value [1,2]. Inevitably, excessive synthetic fertilizers application has resulted in many environmental problems, such as aggravating and accelerating soil acidification [1,3], leaching risk [4], loss of soil microbial diversity [5] and so on. However, in recent years, applying organic fertilizer in tea plantations has been promoted because organic substitution in tea plantations can significantly mitigate soil acidification,
improve soil fertility, increase soil microbial diversity and maintain tea yield [5–7]. Never-
theless, the application of animal-derived organic fertilizers also has the risk of heavy
metal, antibiotic and pathogen microorganism contamination [8–10]. For example, chicken
manure treatment significantly increased available As, Pb, Cu and Zn concentrations com-
pared to chemical fertilizer treatment in tea plantation [11]. Thus, bio-organic fertilizer
(BOF) has raised much attention because it is pollution-free and contains considerable
beneficial microbes and soil-friendly organic materials [12,13]. After BOF application in
a pigeon pea field, the electrical conductivity and bulk density were drastically reduced
while porosity, organic carbon and water holding capacity were significantly elevated [14].
In a field trial and continuous pot experiments in tomatoes, BOF produced tomato yields
equivalent to those obtained using the 100% chemical fertilizer and improved tomato
quality. This may be due to improved soil fertility and soil microbial activity [15]. The
application of BOF in degraded red soil improved soil nutrients, soil microbial diversity
and plant growth [16].

Soil bacteria have been proven to play vital roles in soil nutrient cycling and are
directly or indirectly involved in nitrogen (N) cycling processes and phosphorus (P) miner-
alization [17,18]. Previous studies have demonstrated that BOF application can significantly
influence soil bacterial communities through the specific action of microbial inoculants
or synergistic interaction with the resident soil bacterial communities, thus impacting
rhizosphere microbial activity and plant growth [19,20]. For instance, BOF application can
dramatically increase plant biomass and fruit yield in pomegranate plants by improving
rhizosphere activity and soil fertility [19]. In addition, a recent study has revealed that
BOF application could stimulate indigenous soil Pseudomonas populations to enhance plant
disease suppression [20]. Moreover, BOF application could also accelerate the metabolism
of specific microorganisms and restore soil’s natural nutrient cycling ability [21–23]. There-
fore, we hypothesized that applying BOF in tea plantations may increase the biomass of tea
plants by improving soil fertility and influencing the soil bacterial function groups.

In addition, the rhizosphere microbiome is considered the second genome of plants
and exerts a critical role in plant growth [24–26]. For example, after inoculation with
Bacillus licheniformis MH48, which was isolated from rhizosphere soil, Camellia japonica
seedling development was improved in coastal lands [27]. The application of BOF in
potato field experiments reduced the use of chemical fertilizers and promoted potato
growth [28]. Studies have also shown that the nutrient preference of plants can significantly
alter their rhizosphere microbiome [29]. Tea plants prefer absorbing ammonia to maintain
their growth, which will result in rhizosphere acidification [30]. However, whether the
ammonia preference of tea plants will influence the rhizosphere microbiome is still an open
question. In the meantime, some studies have proved that long-term fertilization could
change the rhizosphere microbiome. For example, recent studies have reported that long-
term fertilization rather than plant species shapes the rhizosphere microbiome and will
reduce the dependence of the rhizosphere microbiome on plant-derived carbon [31,32]. In
previous studies, soil pH has also been proven to be one of key determinant of soil microbial
diversity [17,33]. High N fertilization reduced the diversity of soil fungi and shifted
community composition in tea gardens. These changes were partially due to alterations
in soil pH [34]. Nevertheless, whether BOF application can manipulate the rhizosphere
bacterial community in tea plantation soils remains unclear. Thus, understanding the
effects of BOF application on rhizosphere bacterial communities remains a pressing need.
We hypothesized that BOF application could shape the rhizosphere bacterial communities
through the ammonia preference of tea plants will result in a strong environmental filter
because of the low pH.

In this study, a three-year pot experiment was conducted to investigate (1) how soil
fertility and tea biomass respond to BOF application; (2) how the bulk soil bacterial commu-
nity responds to BOF application, and (3) whether BOF application can alter rhizosphere
bacterial community composition.
2. Materials and Methods

2.1. Experimental Design and Sample Collection

The pot experiment consisted of four different fertilization regimes: control without fertilization (CK), 100% chemical fertilizer (CF), 50% chemical fertilizer + 50% BOF (CFOF) and 100% BOF (OF). The fertilization scheme of each treatment is shown in Table 1.

Table 1. Different experimental groups and fertilizer dosages.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fertilization Regime</th>
<th>Amount of Fertilizer Application (g/Basin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>No fertilizer</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>100% Chemical fertilizer</td>
<td>Urea 4.67</td>
</tr>
<tr>
<td>CFOF</td>
<td>50% Chemical fertilizer + 50% Bio-organic fertilizer</td>
<td>Urea 2.34 + Bio-organic fertilizer 53.75</td>
</tr>
<tr>
<td>OF</td>
<td>100% Bio-organic fertilizer</td>
<td>Bio-organic fertilizer 107.5</td>
</tr>
</tbody>
</table>

The total nitrogen application rate of each treatment was the same (calculated as pure N), which was 350 kg/hm².

One-year-old cutting seedlings of *Camellia sinensis* cultivar ‘Longjing 43’ were selected for this study. The seedlings were planted in 3-gallon plastic flowerpots (the upper diameter, lower diameter and height of pots were 28 cm, 23 cm and 25 cm, respectively). The fertilization amount was calculated according to the area of the upper diameter. In addition to CK, 2.15 g of pure N per flowerpot was applied to each treatment (the total N amount was equivalent to 350 kg/hm²). The N content of BOF was determined to be 2%, and the N content of urea was 46%. The amount of urea and BOF was calculated according to the N content. Four seedlings were planted in each flowerpot, and each treatment included four flowerpots.

The soil was taken from a newly reclaimed tea garden in the Jiangsu Academy of Agricultural Sciences. The properties of the soil are listed in Table 2.

Table 2. The physicochemical properties of soil.

<table>
<thead>
<tr>
<th>Index</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil type</td>
<td>yellow-brown soil</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>5.99</td>
</tr>
<tr>
<td>Total N (g/kg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>13.3</td>
</tr>
<tr>
<td>Available P (mg/kg)</td>
<td>141.6</td>
</tr>
<tr>
<td>Available K (mg/kg)</td>
<td>183.5</td>
</tr>
</tbody>
</table>

BOF was firstly mixed with soil before planting tea seedlings in flowerpots. A week later, urea, P and K fertilizer (K₂HPO₄ 1.0 g/pot), Mg fertilizer (MgSO₄·7H₂O 1.0 g/pot) and Zn fertilizer (ZnSO₄·7H₂O 0.25 g/pot) were dissolved in water and applied in each flowerpot. After application, tea seedlings were placed in the greenhouse. The experiment was started in the middle of April 2016. Fertilization was carried out in April 2017 and 2018 according to the above scheme. The experiment ended in May 2019, and then tea roots were carefully uprooted from the pot and slightly shaken to remove the soil loosely combined with the root. The soil closely adhered to the tea root system was taken as the rhizosphere soil. Other soil was taken as the non-rhizosphere soil. One part of the rhizosphere soil was air-dried and grounded for analysis of the soil’s physical and chemical properties. The other part of the fresh rhizosphere soil was frozen at −80 °C for determination of the soil’s inorganic nitrogen and microbial high-throughput sequencing.

The tea seedlings in each pot were separated by root, stem and leaf, and then the fresh weight of each part was measured by a scale.

2.2. Soil Property Analysis and Soil Fertility Calculation

Soil pH was determined in a 1:2.5 (w/v) soil:deionized water suspension with a pH meter (ORION 3 STAR, Thermo Fisher, Waltham, MA, USA). Soil organic carbon (SOC)
and total nitrogen (TN) were measured by an element analyzer (Vario Max, Elementar, Hanau, Germany). Soil organic matter (SOM) was calculated from SOC by a conversion factor of 1.724. The soil ammonium and nitrate were extracted by 2 M KCl solution, and concentrations in the extracts were determined by the flow injection analyzer (SAN++, Skalar, Breda, The Netherlands). The available P, K, Mg and Ca in the soil were extracted using the Mehlich 3 method [35] and then measured using an inductive coupled plasma emission spectrometer (ICAP6300, Thermo Fisher, Waltham, MA, USA). The soil chemical properties mentioned in this paragraph were conducted following the methods in the soil analysis handbook [36].

The soil fertility index in this study was calculated according to our previous study. The present study used the total data set (TDS) to calculate the soil fertility index. Briefly, the weight of each soil factor was calculated, and then the score of each soil factor was calculated using the standard score function [37]. Finally, the fertility index was calculated by using the following equation:

\[
\text{Fertility index} = \sum_{i=1}^{n} Wi \times Si
\]

where \(Wi\) is the weight value of each soil parameter, \(Si\) is the score of each parameter, and \(n\) is the number of parameters in the TDS [38].

2.3. DNA Extraction, High-Throughput Sequencing

Total soil DNA was extracted from 0.25 g of fresh soil by the DNA Isolation Kit (PowerSoil, MOBIO, Carlsbad, CA, USA) according to the product’s protocol. The quality of extraction was tested by the DNA concentration measurement using a nano spectrophotometer (ND2000, Thermo Scientific, Waltham, MA, USA).

The bacterial 16s rRNA (V4-V5 region) was amplified using the primers 515F/907R [39]. The PCR reaction mixture (25 µL) contained 1 µL of the purified template DNA, 2.5 µL of 10 × PCR Mg\(^{2+}\) free buffer, 2.0 µL of 25 mM dNTPs, 2.5 µL of 2.5 mM Mg\(^{2+}\), 0.5 µL (10 µM) of each primer, and 0.5 µL (1.25 U) of Taq polymerase, and sterilized ultrapure water up to 25 µL. The bacterial V4-V5 region amplification started with an initial denaturation at 94 °C for 5 min, 15 cycles of 94 °C for 60 s, 54 °C for 30 s, 72 °C for 90 s, and a final extension step at 72 °C for 10 min. The PCR was performed by a Thermal Cycler (ABI 2720, Thermo Fisher Scientific, Waltham, MA, USA). The PCR products were then purified with a Gel Extraction Kit (QIAquick, Duesseldorf, Germany).

The purified PCR products were sequenced using the high throughput sequencing platform (MiSeq PE250, Illumina, San Diego, CA, USA). Raw sequence data were deposited in the National Center for Biotechnology Information (NCBI) database with the accession number PRJNA831366.

2.4. Bioinformatics and Statistical Analysis

USEARCH (v 11.0.667) was employed to process the raw bacterial data [40]. In brief, paired raw sequences were merged and re-oriented by comparing them to the RDP database [41]. Then sequences with expected error > 1 and lengths < 250 bp were discarded. Next, fastx_uniques and Unosie3 commands were implemented to remove redundant sequences and chimeras, and representative sequences were obtained in this step. The otutab command was employed to generate the ZOTU table. The representative sequences were aligned against the RDP database with a cutoff value of 0.97 by using the sintax command. The ZOTU table with taxonomic information was used to align against the FAPROTX database using a python script to predict metabolic or other ecologically relevant functions (e.g., nitrification, denitrification or fermentation) [42]. In order to compare the relative difference between samples, a randomly selected subset of 24,900 sequences per sample was performed for downstream analyses.

Bacterial Chao1 and Shannon indices were calculated by using the alpha_div command in USEARCH. The differences in bacterial Chao1 and Shannon indices among treatments
were detected by Kruskal–Wallis’s rank-sum test at \( p < 0.05 \). Principal coordinates analysis (PCoA) was performed using Bray–Curtis distance to evaluate the overall differences in bacterial community structure under different treatments and different compartments, and one-way permutational analysis of variance (PERMANOVA) was used to analyze the effects of treatments and compartments on the community structure of bacterial by using the function “adonis” in the R package “vegan”. The relative abundance of the bacterial community was displayed at the phylum level, and the Proteobacteria was divided into Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria, and other sub-divisions were classified as Proteobacteria. Random forest (RF) analysis was employed to detect the impacts of soil properties on bacterial community composition in the R packages “randomForest”, “rfPermute”, and “rfUtilities”. STAMP was implemented to test the differences in functional profiles between bulk and rhizosphere in BOF treatments and between no BOF input treatments and BOF input treatments. Co-occurrence network analysis was performed in the R package “igraph” to detect the effects of BOF input on bulk and rhizosphere bacterial co-occurrence networks. The visualization of co-occurrence was performed by Gephi (v 0.9.2).

A partial least squares path model (PLS-PM) analysis was applied by using the R package “plspm” to investigate the possible causal relationships between organic input, soil pH, fertility, plant biomass, bacterial community and diversity. Differences in soil properties, plant biomass and soil fertility index among treatments were tested by LSD t-test at \( p < 0.05 \).

All the statistical analysis in the present study was performed in R software (version 4.1.1, R Development Core Team, https://www.r-project.org/, accessed on 7 August 2022).

### 3. Results

#### 3.1. Bulk and Rhizosphere Soil Properties, Fertility and Tea Biomass Response to Different Fertilization Treatments

Our results found that in both bulk and rhizosphere soils, soil pH decreased sharply in CF treatment compared to CK treatment (\( p < 0.05 \)), while CFOF and OF treatments showed mitigation of soil acidification compared to CF treatment (Table 3). In addition, the measured soil properties, including Ca, AK, AP, NO\(_3\)-N, TN and SOM, revealed relatively higher values in CFOF and OF treatments than in CF treatment (Table 3). However, several soil properties, such as soil pH, TN, NH\(_4\)^+ -N and available Ca, displayed relatively higher values in CK treatment compared to those fertilization treatments in bulk soils (Table 3). The soil fertility index of bulk and rhizosphere soils showed a similar variation, i.e., CF displayed the lowest soil fertility index and was significantly lower than CK, CFOF and OF treatments (\( p < 0.05 \)), while BOF application (CFOF and OF) increased soil fertility both in both bulk and rhizosphere soils (Figure 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>AK (mg/kg)</th>
<th>AP (mg/kg)</th>
<th>NH(_4)^+ -N (mg/kg)</th>
<th>NO(_3)^- -N (mg/kg)</th>
<th>pH</th>
<th>SOM (g/kg)</th>
<th>TN (g/kg)</th>
</tr>
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<tbody>
<tr>
<td>Bulk soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>4154.8 ± 419.45 a</td>
<td>382.22 ± 7.91 a</td>
<td>205.35 ± 5.76 c</td>
<td>167.40 ± 6.95 a</td>
<td>14.76 ± 2.28 ab</td>
<td>39.24 ± 1.48 cd</td>
<td>5.57 ± 0.06 a</td>
<td>17.06 ± 0.15 ab</td>
<td>1.45 ± 0.03 a</td>
</tr>
<tr>
<td>CF</td>
<td>3030.5 ± 407.57 bc</td>
<td>488.10 ± 30.68 abcd</td>
<td>205.32 ± 6.10 c</td>
<td>167.40 ± 6.95 a</td>
<td>14.76 ± 2.28 ab</td>
<td>39.24 ± 1.48 cd</td>
<td>5.57 ± 0.06 a</td>
<td>17.06 ± 0.15 ab</td>
<td>1.45 ± 0.03 a</td>
</tr>
<tr>
<td>CFOF</td>
<td>3815.0 ± 179.04 abc</td>
<td>472.06 ± 47.17 ab</td>
<td>205.32 ± 10.14 ab</td>
<td>167.40 ± 6.95 a</td>
<td>14.76 ± 2.28 ab</td>
<td>39.24 ± 1.48 cd</td>
<td>5.57 ± 0.06 a</td>
<td>17.06 ± 0.15 ab</td>
<td>1.45 ± 0.03 a</td>
</tr>
<tr>
<td>OF</td>
<td>3244.0 ± 294.03 abc</td>
<td>472.06 ± 47.17 ab</td>
<td>205.32 ± 6.10 c</td>
<td>167.40 ± 6.95 a</td>
<td>14.76 ± 2.28 ab</td>
<td>39.24 ± 1.48 cd</td>
<td>5.57 ± 0.06 a</td>
<td>17.06 ± 0.15 ab</td>
<td>1.45 ± 0.03 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rhizosphere soil</th>
<th>Ca (mg/kg)</th>
<th>Mg (mg/kg)</th>
<th>AK (mg/kg)</th>
<th>AP (mg/kg)</th>
<th>NH(_4)^+ -N (mg/kg)</th>
<th>NO(_3)^- -N (mg/kg)</th>
<th>pH</th>
<th>SOM (g/kg)</th>
<th>TN (g/kg)</th>
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</thead>
<tbody>
<tr>
<td>CK</td>
<td>4124.6 ± 224.1 ab</td>
<td>414.38 ± 4.04 bcd</td>
<td>205.40 ± 17.14 b</td>
<td>162.15 ± 6.66 ab</td>
<td>8.52 ± 0.45 c</td>
<td>38.99 ± 3.58 cd</td>
<td>5.54 ± 0.04 a</td>
<td>17.42 ± 0.32 ab</td>
<td>1.05 ± 0.09 b</td>
</tr>
<tr>
<td>CF</td>
<td>2806.4 ± 352.2 c</td>
<td>472.06 ± 20.88 ab</td>
<td>205.40 ± 13.15 b</td>
<td>162.15 ± 6.66 ab</td>
<td>8.52 ± 0.45 c</td>
<td>38.99 ± 3.58 cd</td>
<td>5.54 ± 0.04 a</td>
<td>17.42 ± 0.32 ab</td>
<td>1.05 ± 0.09 b</td>
</tr>
<tr>
<td>CFOF</td>
<td>3094.4 ± 179.0 abc</td>
<td>490.40 ± 9.72 c</td>
<td>205.40 ± 13.15 b</td>
<td>162.15 ± 6.66 ab</td>
<td>8.52 ± 0.45 c</td>
<td>38.99 ± 3.58 cd</td>
<td>5.54 ± 0.04 a</td>
<td>17.42 ± 0.32 ab</td>
<td>1.05 ± 0.09 b</td>
</tr>
<tr>
<td>OF</td>
<td>2014.6 ± 436.82 bc</td>
<td>490.40 ± 9.72 c</td>
<td>205.40 ± 13.15 b</td>
<td>162.15 ± 6.66 ab</td>
<td>8.52 ± 0.45 c</td>
<td>38.99 ± 3.58 cd</td>
<td>5.54 ± 0.04 a</td>
<td>17.42 ± 0.32 ab</td>
<td>1.05 ± 0.09 b</td>
</tr>
</tbody>
</table>

CK, control treatment; CF, 100% chemical fertilizer; CFOF, 50% chemical fertilizer + 50% bio-organic fertilizer; OF, 100% bio-organic fertilizer. Different letters in the same column represent significant differences among treatments at a significance level of \( p < 0.05 \).

The biomasses of roots and total plant were significantly increased after fertilization compared to CK treatment (\( p < 0.05 \), and no significant difference was found between CF, CFOF and OF treatments (Figure 2b,c). However, leaf biomass displayed the highest
value in CFOF treatment and was significantly higher than that in CK and CF treatments ($p < 0.05$) (Figure 2a).

**Figure 1.** Soil fertility index in bulk and rhizosphere soil under different fertilization treatments. CK, control treatment; CF, 100% chemical fertilizer; CFOF, 50% chemical fertilizer + 50% bio-organic fertilizer; OF, 100% bio-organic fertilizer. Different letters in rows indicated the significant differences at $p < 0.05$.

**Figure 2.** Biomass of leaf (a), root (b) and total tea plants (c) as affected by different fertilization treatments. CK, control treatment; CF, 100% chemical fertilizer; CFOF, 50% chemical fertilizer + 50% bio-organic fertilizer; OF, 100% bio-organic fertilizer. Different letters on each bar represent significant differences among treatments at a significance level of $p < 0.05$. 
3.2. Bulk and Rhizosphere Soil Bacterial Diversity and Community Composition Change under Different Fertilization Treatments

In the present study, the bacterial Chao1 index in bulk soils did not show significant differences among different fertilization treatments, whereas significant differences were found in rhizosphere soils (Figure 3a). In addition, the Chao1 index increased after fertilizer input decreased with the increasing amount of BOF input (Figure 3a). The Shannon index in rhizosphere soils showed the same variation trend as the Chao1 index in bulk soils and displayed significant differences among treatments (Figure 3b).

![Figure 3](image)

**Figure 3.** Alpha-diversity indices including Chao1 (a) and Shannon (b) in bulk and rhizosphere soil bacteria. Bacterial community structure (c) and composition (d) change under different fertilization treatments. CK, control treatment; CF, 100% chemical fertilizer; CFOF, 50% chemical fertilizer +50% bio-organic fertilizer; OF, 100% bio-organic fertilizer. Different letters in rows indicated significant differences at $p < 0.05$.

The bacterial community composition showed that Betaproteobacteria, Acidobacteria, Alphaproteobacteria and Gammaproteobacteria were the dominant phyla in this study, with an average relative abundance of 46.88%, 11.29%, 9.69% and 9.59%, respectively (Figure 3d). Moreover, the relative abundance of Betaproteobacteria, Acidobacteria, Alphaproteobacteria, Planctomycetes, Deltaproteobacteria and Gemmatimonadetes displayed a significant change among treatments in bulk soils. In rhizosphere soils, only the relative abundance of Actinobacteria and Gemmatimonadetes was significantly changed among treatments (Figure S1). Specifically, CFOF and OF treatments notably increased the relative abundance of Alphaproteobacteria.
teobacteria, Acidobacteria, Deltaproteobacteria and Planctomycetes while significantly decreasing the relative abundance of Betaproteobacteria (Figure S1).

Bacterial community structure also displayed a notable difference among treatments (PERMANOVA test: $R^2 = 0.43$, $p < 0.001$) (Figure 3c). The RF analysis showed that soil properties explained 74.91% of the community change ($R^2 = 0.74$, $p < 0.001$), and soil available K (24.1%, $p < 0.01$), Ca (15.3%, $p < 0.01$), P (12.1%, $p < 0.05$), Mg (10.5%, $p < 0.05$), pH (14.4%, $p < 0.05$) and total N (8.53%, $p < 0.05$) contributed significantly to the bacterial community change (Figure 4). The remaining soil properties made little contribution to the bacterial community change.

Figure 4. The impacts of soil properties on bacterial community composition by RF analysis. Soil properties are ranked in ascending order of importance to the accuracy of the model. The significance level is indicated by ** ($p < 0.01$) and * ($p < 0.05$).

3.3. Bacterial Function Group and Co-Occurrence Network Response to BOF Addition

To detect the effect of BOF input on bacterial functional group and co-occurrence network, we categorized two new treatments, i.e., no BOF (NBOF) input treatment (including CK and CF treatments) and BOF input treatment (including CFOF and OF treatments), to explore the response of the bacterial function group and co-occurrence network to BOF addition.

For the BOF treatment, the FAPROTAX functional predictions identified 18 differentially present elements cycling between bulk and rhizosphere soils in BOF treatment. Most functions had higher proportions in bulk soils except for ureolysis and N fixation (Figure 5a). The largest significant differences were functions for nitrification, methanol oxidation, methylotrophy, intracellular parasites, ureolysis and N fixation (Figure 5a). In addition, 22 differentially functions were identified between BOF and NBOF in bulk soils, and 18 functions had higher proportions in BOF treatments (Figure 5b). The largest significant differences were functions for chemoheterotrophy, intracellular parasites, nitrification, methanol oxidation, methylotrophy, ureolysis and N fixation (Figure 5b).
Figure 5. Functional predictions of bacterial communities between bulk and rhizosphere soils under different fertilization treatments. (a) Comparison between bulk and rhizosphere soils in BOF treatment; (b) comparison between BOF and NBOF in bulk soil bacteria.
The bacterial co-occurrence networks were distinctly different in bulk and rhizosphere soils and in BOF and NBOF treatments (Figure 6 and Table 4). Our results found both BOF and NBOF treatments, the bacterial co-occurrence networks in bulk soils revealed more nodes and edges than in rhizosphere soils, and with relatively higher average degrees, average path lengths and diameters (Table 4), which indicated that bulk soils had more complex networks than rhizosphere soils. In bulk soils, the co-occurrence network nodes and edges in BOF treatment (nodes = 154, edges = 281) increased compared to NBOF treatment (nodes = 97, edges = 226), while the proportion of negative edges in BOF treatment revealed a dramatic decrease compared to NBOF (from 34.51% to 13.17%) (Figure 6a,b; Table 4). However, in the rhizosphere, BOF treatment displayed a simpler co-occurrence network compared to NBOF treatment, and all the network properties were decreased in the BOF treatment except for the density and modularity (Figure 6c,d; Table 4).

**Figure 6.** The bacterial co-occurrence networks in bulk soil of NBOF ((a): CK and CF) and BOF ((b): CFOF and OF) treatments, in rhizosphere soils of NBOF ((c): CK and CF) and BOF ((d): CFOF and OF) treatments. Node size is proportional to the betweenness centrality of each genus, and edge thickness is proportional to the weight of each correlation. The color of each edge represents positive and negative correlation coefficients: red represents positive correlation, and blue represents negative correlation. The thickness of each edge is proportional to the correlation coefficient ($p < 0.01$).
3.4. Bacterial Diversity, Community Structure, Soil pH, Fertility and Tea Biomass Response to BOF Input

A PLSPM analysis was employed to detect the complex relationships among soil pH, fertility, bacterial community diversity, structure, and tea biomass under BOF treatments. The model showed that BOF input explained 47% of the variance in biomass with a 0.59 goodness of fit (Figure 7). The results showed that BOF input had positive and direct effects on tea biomass (path coefficient (pc) = 0.56), soil fertility (pc = 0.58) and bacterial community structure (pc = 0.31) but also showed direct negative effects on bacterial diversity (pc = −0.12). In addition, bacterial community structure negatively affected tea biomass (pc = −0.19), while bacterial diversity displayed an opposite result (pc = 0.40). Soil fertility (pc = 0.09) and pH (pc = −0.09) revealed slightly direct effects on tea biomass (Figure 7a); however, soil pH also exhibited an indirect effect (pc = −0.19) on tea biomass (Figure 7b). Excluding the indirect negative effect, the total effect of BOF input was 0.48 (Figure 7b).

![Figure 7](image_url)

**Figure 7.** The relationship between bacterial diversity, community structure, soil pH, fertility, BOF input and tea biomass. (a) Path model outputs: the numbers on the arrows represent standardized path coefficients. The value of each path coefficient is indicated by the arrow width; blue and red arrows indicate positive and negative effects, respectively. Values of R² indicate the variance explained by the model. (b) The standardized path coefficients for direct and indirect effects on the bacterial community.
4. Discussion

4.1. Effect of BOF Application on Soil Properties and Fertility

In the present study, BOF application (CFOF and OF treatments) revealed an excellent offset effect on soil acidification and a notable improvement in soil fertility compared to CF treatment (Table 3 and Figure 1). These results are consistent with previous studies and have been verified in many field experiments with different plants, such as pigeon pea [14], potatoes [15] and grains [16]. However, CF treatment displayed the lowest soil fertility, even lower than CK treatment. We suggested that the strong leaching effect under pure chemical fertilizer treatments might be the reason for the decrease in soil fertility [43]. Many studies have proved that pure chemical fertilizer input can increase the leaching risk, while chemical fertilizer combined with organic amendments (e.g., organic fertilizer, straw and biochar) has good performance in reducing soil nutrient leaching [44,45]. In addition, the rhizosphere displayed a relatively lower soil pH than the bulk soils. This result could be attributed to the preference for ammonia absorption and the organic acids in root exudates because both of these could acidify the root-zone pH [46,47].

4.2. Effect of BOF Application on Soil Bacterial Diversity, Structure, Function Group and Co-Occurrence Network

Previous research has proved that applying BOF to fields has tremendous influences on soil bulk and rhizosphere bacterial communities. For example, applying BOF can increase soil bacterial diversity in tobacco, potato and cucumber soils compared to pure chemical fertilization treatment [28,48,49]. However, the soil bacterial Chao1 index in bulk did not show significant change after applying BOF, and only the Shannon index showed a slight increase in BOF treatment (Figure 3a,b). We suggested that the short term of this experiment might be the reason for this result because active microbes in BOF may compete with the local bacterial community and result in a decline in richness [50,51]. In addition, the bacterial Chao1 and Shannon index showed a significant decrease under BOF treatments (CFOF and OF) in the rhizosphere. Previous studies have reported that applying BOF can promote the formation of beneficial microbial communities in the rhizosphere, which may also lead to a decrease in microbial diversity because of the harbouring of beneficial microbes [52]. The co-occurrence network also confirmed that, i.e., the network of BOF treatment in the rhizosphere showed fewer nodes and edges (including negative edges) (Figure 6c,d).

Nevertheless, bulk soil bacterial composition was significantly changed under different treatments, although rhizosphere soil bacterial composition showed no significant change for most phyla, indicating that the rhizosphere effects intensely impact bacterial composition [53,54]. Moreover, Proteobacteria and Acidobacteria dominated at the phylum level. This result was similar to previous studies in acidic soils [7,55]. Acidobacteria was reported that have the function of C cycling through degradation [56,57]. BOF treatments significantly increased Acidobacteria relative abundance compared to CF treatment, which indicated that BOF could improve the function of C degradation. The functional prediction results further proved that BOF input did enhance the aromatic hydrocarbon and hydrocarbon degradation processes compared to NBOF treatment (CK and CF treatments) (Figure 5b). Proteobacteria constitute the most dominant fraction of the mangrove bacterial community. All classes of Proteobacteria (Alpha, Beta, Gamma, Delta, and Epsilonproteobacteria) have been reported from mangroves across the globe, although the abundance of different classes varied significantly [58–60]. Since Alphaproteobacteria can convert atmospheric N to nitrates, making nitrogen usable by other forms of life, the increase in the relative abundance of Alphaproteobacteria in BOF treatments indicated that BOF had a positive effect on N transform processes. Moreover, the functional prediction results again verified that nitrification and denitrification processes were enhanced in BOF treatments. Deltaproteobacteria are considered to have hydrocarbon-degrading abilities [61]. The increase in the relative abundance of Deltaproteobacteria in BOF treatments indicated that BOF could increase C degradation. In addition, the contributions of soil properties to bacterial community structure changes
in the present study were similar to our former study in tea plantations, i.e., soil nutrients and pH change can remarkably change the bacterial community structures [5,62]. After organic fertilizer substitution in a tea plantation for ten years, the soil pH ($p = 0.005$) and SOC ($p = 0.017$) were the predominant soil characteristics that accounted for the structural changes in the soil bacterial community [5]. In an eleven-year field experiment with different N applications, the variation of bacterial community composition was largely explained (~50%) by the soil properties of pH, exchangeable magnesium, exchangeable potassium and exchangeable hydrogen [62].

BOF application usually has significant positive effects on suppressing pathogens to prevent plants from contracting diseases in previous studies [48–50]. In addition, BOF application also revealed notable influences on soil bacterial functional profiles in previous studies [16,23]. For instance, carbohydrate/lipid metabolism and the biosynthesis of other secondary metabolites were improved after BOF addition in banana plantations [23]. Moreover, the application of BOF in degraded red soil also revealed positive impacts on N cycling, enhanced recalcitrant C degradation, and inhibited labile C degradation [16]. These results were similar to ours, i.e., BOF significantly enhanced N cycling and C degrading processes (Figure 5). Interestingly, our results also showed that rhizosphere soil ureolysis and N fixation processes were enhanced compared to bulk soil after the addition of BOF. This result is in line with the recent research showing that genes involved in organic compound conversion and nitrogen fixation were strongly enriched in the rhizosphere [63].

Previous studies showed that when organic fertilizer was added to soils, a more complex co-occurrence network developed than with the addition of inorganic fertilizer [64,65]. A similar result was found in our study, where the bacterial network modularity and negative edges increased with the OSRs (Figure 4 and Table 3). However, the bacterial co-occurrence network in the rhizosphere soil displayed a reverse result; we suggested that the bacterial community in the rhizosphere was more affected by the rhizosphere effect than fertilization [53,54].

4.3. Effect of the Application of BOF on Improving Tea Biomass

It is well demonstrated that the application of BOF could improve soil fertility, suppress soil-borne diseases in agricultural production, and increase crop yields or plant biomass [28,49]. For example, BOF application decreased disease incidence of tobacco bacterial wilt and cotton Verticillium wilt; contributing factors to this were the reduction of the abundance of potentially pathogenic microbes and the recruitment of more beneficial bacteria or fungi in rhizosphere soil, thus improving pathogenic bacteria resistance and promoting plant growth [48,52]. In the present study, we found that BOF substituted for 50% of chemical fertilizer displayed the highest leaf and total biomasses. This result was similar to the recent research, indicating that partial substitution of chemical fertilizers with BOF is a promising fertilization practice for banana production in acid soil ecosystems [23].

In addition, PLSPM analysis showed that BOF input and bacterial diversity have direct positive effects on tea biomass. This result could be attributed to the improvement of soil fertility and bacterial functions. A previous study also found a similar result because BOF input could increase soil fertility and enhance the bacterial function profiles in tea plantation soils [66]. However, soil fertility showed a negligible positive effect on tea biomass in our study. We suggested that fertilization treatments could supply adequate nutrients to maintain the tea plant growth in such a short-term pot experiment, though CF treatment had a lower soil fertility index because of the leaching. Therefore, total plant biomass showed no difference between fertilization treatments after a 3-year experiment, which might be the reason for the negligible positive effect on tea biomass in our study.

5. Conclusions

Overall, the present study proved that BOF application could increase soil fertility in both bulk and rhizosphere soils and improve the biomass of tea leaves. In addition, the nutrient level changes caused by BOF application significantly changed bacterial com-
Community diversity and composition and accounted for 74.91% of the community variation. Furthermore, BOF notably improved the N and C cycling processes and enhanced the co-occurrence network complexity in the bulk soils, whereas bacterial community functions in the rhizosphere were more affected by the rhizosphere effect than BOF application. All these findings verified our hypothesis that applying BOF in tea plantations could increase the biomass of tea plants by improving soil fertility and influencing the soil bacterial function groups. In addition, BOF application could influence the rhizosphere bacterial communities, but the rhizosphere effects affect more.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092168/s1, Figure S1. Variance analysis of the top 9 bacterial relative abundance in bulk and rhizosphere soil in phylum level.

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