The Response of Soil Nutrients and Microbial Community Structures in Long-Term Tea Plantations and Diverse Agroforestry Intercropping Systems

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Abstract: During tea cultivation, diverse agroforestry is an important and established intercropping measure, with most studies concentrating on ecological service provision and economic returns. However, the response of soil nutrients and microbial community structures in long-term tea plantations with diverse agroforestry intercropping systems is poorly understood. In the present field study (2015), three intercropping agroforestry-tea patterns (Osmanthus-Tea (OT), Michelia-Tea (MT), Osmanthus-Michelia-Tea (OMT)) along with a study control (C) were examined in terms of these two knowledge gaps. Results showed that, in terms of tea cultivation, the OMT system is more suitable than the OT and MT systems. The OMT system significantly increased the total nitrogen (TN, 16.4%), total potassium (TK, 10.5%), available nitrogen (AN, 14.2%), available phosphorus (AP, 26.7%) and soil organic matter (SOM, 28.9%). The OMT system increased phylum *Firmicutes* and *Bacteroidetes* abundance by 35.8% and 9.6%. In addition, the OMT system enhanced the abundance of class *Actinobacteria* (99.5%), *Erysipelotrichia* (96.9%), *Clostridia* (93.5%) and *Actinobacteria* (19.6%), respectively. In general, the phylum bacteria *Proteobacteria*, *Firmicutes*, *Actinobacteria* accounted for the largest proportion of bacteria in all three intercropping systems. In this study, the abundance of *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* were positively correlated with AN, SOM and TP. The results of the present study will help to develop a better understanding of the benefits imposed by different agroforestry intercropping systems on nutrient dynamics and microbial structural diversity during tea cultivation.

Keywords: *Camellia sinensis*; intercropping system; microbial diversity; soil nutrients

1. Introduction

Tea (*Camellia sinensis*) belongs to perennial evergreen woody plants and is widely distributed in tropical and subtropical regions [1,2]. Previous research has reported that tea plantations could improve biological properties and enhance the content of SOM and nutrients, microbial basal respiration, biomass carbon and N in soil [3–6]. However, long-term tea monoculture can decrease the metabolic quotient, microbial diversity, and beneficial bacteria [2,7,8]. In a 20-year continuous tea cropping study, *Pseudomonas*, *Rhodanobacter*, *Bradyrhizobium*, *Mycobacterium* and *Sphingomonas* and other beneficial bacteria were significantly decreased [8,9]. In addition, *Mortierella alpina* and *Mortierella elongatula* and other beneficial fungal species were decreased, while *Fusarium oxysporum*, *Fusarium solani*, and *Microdium phyllanthi* and other potentially pathogenic fungal species were increased in...
continuous tea cropping systems [10]. Beneficial bacteria play an important role in the soil nutrient conversion cycle, sustainable utilization, and anti-interference ability [11–13]. Moreover, long-term tea monocropping may cause change in soil structure and mineral depletion. For instance, Iori et al. [14] reported that the degradation in soil physical, chemical and biological properties decreased the production output of older tea plantations. Similarly, Gogoi et al. [15] showed that on a 50-year-old tea monocropping plantation, an imbalance of soil nutrients was found during different seasons.

Plant species and intercropping treatments can largely affect the soil properties and the composition of the microbial community [16]. Diverse agroforestry-tea intercropping systems of two or more crops have been employed for tea production [17]. It has been well documented that agroforestry can enhance and maintain productivity and sustainability in intercropping systems [18–20]. In Asia, the use of agroforestry to provide shade in tea plantations is gaining popularity [21]. For example, Wu et al. [22] found that rubber-tea intercropping can not only redistribute water sources well underground in this agroforestry system, but can also maintain plant root health underneath the soil of the terrace. Similarly, Ma et al. [23] showed that Castanea mollissima Blume-tea intercropping can improve tea quantity and quality. Mortimer et al. [24] have proven that alder trees (Alnus nepalensis) in tea plantations promote the growth and development of the soil microbial communities. Overall, agroforestry providing shade has the potential to improve soil properties and bacteria biodiversity, while attaching economic value.

Some studies have selected suitable plant species for intercropping with tea bushes, which relieve long-term problems associated with monoculture [25]. Michelia (Michelia chapensis Dandy) is an expensive broad-leaved timber species [26,27]. Similarly, Osmanthus (Osmanthus fragrans Lour.) is a commonly used evergreen aromatic ornamental tree [28]. Diversity of species in an intercropping system could enhance the use of resources which is based on the compatibility and the complementary nature of ecology services. Amongst these beneficial roles, positive and negative bacteria play key roles in tea plant growth and production [29]. Rhizosphere microbial communities largely result in positive interactions within the intercropping system, while negative effects may be driven by the soil microbial being reinforced by competing with plants for nutrients [30]. Bacterial microbial communities provide more integrative information regarding soil environmental changes compared with SOM decomposition and soil nutrient cycling, as well as soil structure formation and stability [31–33]. Current research focuses on the impact of agroforestry on soil communities, which does not always lead to an increase in the microbial diversity [34]. Thus, little information is available on the impact that diverse agroforestry systems used in tea cultivation have on soil nutrients and microbial community abundances.

Understanding the response of soil microbial to long-term intercropping is a basic premise for using agroforestry in maintaining tea productivity and soil nutrient sustainability. However, few studies have explored the changes in soil nutrients and the microbial community under different intercropping systems. The objectives of this study were to investigate: (1) the soil nutrient status of the tea plantation soil environment under three different intercropping patterns; (2) how the microbial community structure changes across the diverse intercropping systems; and (3) the correlation between soil microbial community structures and soil nutrient status across the three intercropping systems.

2. Materials and Methods

2.1. Study Sites

This field experiment was conducted at the Experimental Station of the station of Chinese Academy of Sciences in North Hunan Province, China (31°01′ N, 115°53′ E, and at an altitude of 100–200 m) (Figure 1). The mean annual temperature across the four seasons is between 16.8 and 19.5 °C, ranging from 4.5 °C in January to 40.6 °C in July, and the mean annual rainfall is 1437 mm. The soil texture is typically light loamy, with a pH of 4.1–6.4 and depth of 100 cm.
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2.2. Experimental Design

For this field study, a 26-year-old tea garden which had an area of approximately 1300 ha was used. The spacing of the tea bushes was 0.35 × 1.5 m². After 26 years of pure tea (C. sinensis cv. baihaozao) monoculture management, Osmanthus (Osmanthus fragrans Lour.) and Michelia (Michelia chapensis Dandy) trees were intercropped in 2001, and sampled in 2015. Four spatially interspersed sample plots were randomly established for each of the intercropping treatments, while maintaining a control (pure tea bushes without other trees). Each sample plot was 50 × 60 m² in area, located at a distance of at least 10 m between adjacent plots. The spacing of the Osmanthus tree and/or Michelia was at least 8 × 8 m² (Figure 1a,b). For each sample plot, one row of Osmanthus and/or Michelia trees was planted every six rows of tea bushes (Figure 1). In this study, three intercropping systems (1) Osmanthus tree-tea tree (OT); (2) Michelia-tea tree (MT); (3) Osmanthus tree-Michelia-tea (OMT) and a control/pure tea garden were used (Figure 1). All systems underwent identical agronomic management practices.

2.3. Soil Chemical Analysis

In each system, soil samples from the depth of 0–20 cm were extracted, naturally air-dried and then finely ground and sieved through a 2 mm filter. The tea orchard soil samples were collected from the four systems (C, OT, OM and OMT system). Each system consisted of 6 sub-samples, 4 replicates. After collection, the soil samples were placed into a low temperature fridge at −70 °C and immediately transported to the laboratory. In short, the Kjeldahl digestion method was used to extract TN [35]. TP and TK were determined using the sodium carbonate method and NaOH melt flamer, respectively [36]. In addition, AN was measured using the combination pH alkaline hydrolysable method. The hydrochloric acid and flame photometry analyzed the AP and AK, respectively [37]. SOM was measured using the oxidative thermal potassium dichromate oxidation method [38].
2.4. DNA Extraction

DNA was extracted by the OMEGA E.Z.N.A soil DNA kit (OMEGA, USA), according to the manufacturer’s instructions [39]. DNA quality was detected by 1.2% agarose gel electrophoresis.

2.5. Illumina Hiseq Sequencing and Data Analysis

Illumina Hiseq sequencing approach was used to evaluate the abundance and the structure of microbiota in agroforestry-tea intercropping system. To determine the fungal diversity in the intercropping systems, primer (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and reverse primer (5′-GCTGCGTTCTTCATCGATGC-3′), which target the V4 region of ITS1, were selected. Meanwhile, the primers pair, 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) was used for the amplification of the 16S rRNA region to detect the bacterial diversity of the four systems. A DNA library was generated with the purified PCR products via the quantitative DNA binding approach using the TruSeq® DNA PCR-Free Sample Preparation Kit. Afterwards, the DNA library was sequenced on the Illumina HiSeq platform, which was provided by the Beijing Genomics Institute (Shenzhen, China). Connecting Overlapped Pair-end (COPE) software, V 1.2.1, was used to connect the 250 bp pair-end reads, when removing barcodes and adaptor sequences [40]. To control the quality of the raw tags, trimming low-quality sequences using Qiime V1.9.1 [41] was used. The effective tags were used for further analysis, which were obtained after detecting and removing chimera using the UCHIME Algorithm and Unite database, respectively [42,43]. The effective tags into operational taxonomic units (OTUs) were clustered using Uparse software [44]. The typical OTUs blast analysis was annotated by Qiime software and Unit database, respectively [45]. Multiple sequence alignment was conducted by MUSCLE V3.8.31 [46].

2.6. Statistical Analysis

One-way ANOVA was performed to determine the effect of intercropping on the soil nutrient components in these soil samples. Multivariate ANOVA and Duncan’s new complex range method were used to statistically analyze the soil nutrients and the abundance of soil microbial (SPSS IBM20, USA). Spearman’s correlation coefficients were utilized to assess the correlations between soil nutrients and bacterial phyla and class. Variability in the data is stated as the standard deviation (SD), n = 4, when P < 0.05 was considered to be statistically significant.

3. Results

3.1. Change in Soil Nutrients

Overall, except for TP and AK in the soil, significant differences were found between the soil properties of the four systems (Figure 2a–g). Intercropping treatments significantly increased the TN, TK, AN, AP and SOM. with the increased percentages followed the following sequence: OMT system > OT system > MT system > C (Figure 2). The OMT system significantly increased the TN (16.4%, P < 0.05), TK (10.5%, P < 0.05), AN (14.2%, P < 0.05), AP (26.7%, P < 0.05) and SOM (28.9%, P < 0.05) compared with the C system.
respectively, compared to the C system. The abundance of bacteria in the MT system had the following trend:

Proteobacteria (44.6%) > Acidobacteria (16.4%) > Firmicutes (11.4%) > Actinobacteria (10.3%) > Chloroflexi (9.7%) > Bacteroidetes (3.3%).

Intercropping significantly increased Proteobacteria and Firmicutes abundance by 18.4% and 8.8%, respectively, while Actinobacteria and Chloroflexi decreased by 18.0% and 7.9%, respectively, compared to the C system.

**Figure 2.** Comparison of soil nutrients in the four systems: (a) total nitrogen (TN); (b) total phosphorus (TP); (c) total potassium (TK); (d) soil organic matter (SOM); (e) soil available nitrogen (AN); (f) available phosphorus (AP); (g) available potassium (AK). The differences of soil nutrients concentration among the four treatments (control/pure tea garden (C); Osmanthus tree-tea (OT); Michelia-tea (MT) and Osmanthus-Michelia-tea (OMT)) were tested by one-way ANOVA. Bars with different lowercase letters indicate a significant difference ($P < 0.05$) among the four intercropping treatments. All values represent the mean ± standard deviation (SD), $n = 4$.

### 3.2. Soil Phylum Microbial Diversity

Hiseq sequencing data showed that at the phylum level, there were significant differences between the four systems (Figure 3). There was no significant differences among Thermomicrobia, Cyanobacteria, Gemmatimonadetes, and Verrucomicrobia in the four systems,
accounting for less than 1% (Figure 3A,B). In the OT system, the proportion of bacteria followed the following sequence: Proteobacteria (31.8%) > Firmicutes (26.7%) > Actinobacteria (14.4%) > Acidobacteria (13.2%) > Chloroflexi (4.8%) > Bacteroidetes (4.1%). Intercropping significantly increased the Proteobacteria and Firmicutes abundance by 24.1% and 5.8%, respectively, but Actinobacteria and Chloroflexi abundance decreased by 13.9% and 12.9%, respectively, compared to the C system. The abundance of bacteria in the MT system had the following trend: Proteobacteria (44.6%) > Acidobacteria (16.4%) > Firmicutes (11.4%) > Actinobacteria (10.3%) > Chloroflexi (9.7%) > Bacteroidetes (3.3%). Intercropping significantly increased Proteobacteria and Firmicutes abundance by 18.4% and 8.8%, respectively, while Actinobacteria and Chloroflexi decreased by 18.0% and 7.9%, respectively, compared to the C system.

![Relative abundance of soil bacterial communities](image)

**Figure 3.** Relative abundance of soil bacterial communities: (A) The average relative abundance of four systems soil samples’ phylum. Different colors represent the soil phylum bacterial. (B) Class is presented in bar plots showing the microbe variation within the four systems.

Furthermore, in the OMT system, the following trend was observed: Firmicutes (38.4%) > Actinobacteria (15.4%) > Proteobacteria (15.0%) > Bacteroidetes (12.1%) > Acidobacteria (8.6%) > Chloroflexi (7.5%). Intercropping significantly increased Firmicutes and Bacteroidetes abundance by 35.8% and 9.6%, respectively, but decreased Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria by 11.1%, 12.9%, 10.2% and 4.9%, respectively, compared to the C system. Overall, intercropping enhanced the Firmicutes, Proteobacteria and Bacteroidetes compared to the C system. The abundance of Firmicutes in the four treatments followed the trend of OMT (38.4%) > OT (26.7%) > MT (11.4%) > C (2.6%). The abundance of Proteobacteria in the four treatments had the following trend: MT (44.6%) > OT (31.9%) > C (26.1%) > OMT (15.0%). In addition, the abundance of Bacteroidetes in the four treatments were in the following sequence: OMT (12.1%) > OT (4.1%) > MT (3.3%) > C (2.5%).

### 3.3. Abundance of Different Microbial Classes

From the classification level of class (Figure 3), a total of 83 known bacterial classes were obtained. The soil microbes are grouped at the taxonomic class level for all further analyses. These 10 most abundant classes of the sequence are the sum of 61.38% to 85.73%. In the OT system, the 10 most abundant classes followed the following sequence: Alphaproteobacteria (16.0%) > Bacilli (12.7%) > Clostridia (10.7%) > Acidobacteria (9.6%) > Gammaproteobacteria (9.5%) > Actinobacteria (8.8%) > Ktedonobacteria (3.8%) > Erysipelotrichia (3.2%) > Bacteroidia (2.4%) > Acidimicrobia (1.6%).

Tea-tree intercropping could effectively enhance the abundance of Bacteroidia, Erysipelotrichia, Bacilli, Clostridia, Gammaproteobacteria and Acidobacteria by 97.6%, 93.4%, 90.2%, 89.3%, 54.6% and 46.3%, respectively, but the Acidimicrobia, Ktedonobacteria Alphaproteobacteria and Actinobacteria decreased by 466.1%, 177.2%, 21.2% and 16.6%, respectively, compared to the C system.

In the MT system, the order of the 10 most abundant classes were Gammaproteobacteria (22.3%) > Alphaproteobacteria (17.2%) > Actinobacteria (7.8%) > Ktedonobacteria (7.1%) > Acidobacteria (7.0%) > Clostridia (5.4%) > Bacilli (4.3%) > Bacteroidia (2.0%) > Erysipelotrichia (1.7%) > Acidimicrobia (0.7%). Tea-tree intercropping systems were compared with the C system.
system, which indicated that intercropping increased the abundances of Bacteroidia (97.2%), Erpsipelotrichia (87.2%), Gammaproteobacteria (80.5%), Clostridia (78.7%) and Acidobacteria (26.9%), but decreased the abundances of Acidimicrobia (1254.0%), Ktedonobacteria (50.8%), Actinobacteria (31.2%) and Alphaproteobacteria (12.8%).

Furthermore, the 10 most abundant classes in the OMT system had the following trend: Clostridia (17.4%) > Bacilli (13.8%) > Actinobacteria (12.8%) > Bacteroidia (11.2%) > Alphaproteobacteria (7.3%) > Erpsipelotrichia (7.0%) > Ktedonobacteria (6.6%) > Gammaproteobacteria (4.5%) > Acidobacteria (4.4%) > Acidimicrobia (0.7%). Intercropping enhanced the abundance of Bacteroidia (99.5%), Erpsipelotrichia (96.9%), Clostridia (93.5%), Actinobacteria (19.6%), while it decreased the Acidimicrobia, Alphaproteobacteria, Ktedonobacteria and Acidobacteria by 1266.7%, 165.3%, 62.7% and 15.6%, respectively. In the three intercropping systems, Bacteroidia, Erpsipelotrichia, Clostridia and Actinobacteria were increased by tea-tree positive interactions compared to the C system. The abundance of Bacteroidia in the four treatments was in the following sequence: OMT (11.2%) > OT (10.3%) > OT (8.8%) > MT (7.8%). The abundance of Erpsipelotrichia in the four treatments followed the trend of OMT (7.1%) > OT (3.2%) > MT (1.7%) > C (0.2%). The abundance of Clostridia in the four treatments had the following trend: OMT (17.4%) > OT (10.7%) > MT (5.4%) > C (1.1%). In addition, the abundance of Actinobacteria in the four treatments showed the following sequence: OMT (12.8%) > C (10.3%) > OT (8.8%) > MT (7.8%).

### 3.4. Correlation between Bacterial Communities and Soil Nutrients

In Table 1, the effects of soil nutrient factors on the taxonomy of bacteria phyla are as follows: the contents of AN, SOM and TP were significantly positively correlated with Firmicutes (0.47 *, 0.37 *, 0.54 **), Actinobacteria (0.43 *, 0.46 *, 0.32 *), Proteobacteria (0.38 *, 0.56 **, 0.25 *) and Bacteroidetes (0.67 **, 0.27 *, 0.31 *). TN, TK, AK, and AP are significantly negative to Chloroflexi (−0.57 *, −0.49 *, −0.41 *, −0.33 *), Gemmatimonadetes (−0.26 *, −0.19 *, −0.72 **, −0.31 *), Acidobacteria (−0.86 **, −0.79 *, −0.86 **, −0.39 **), Verrucomicrobia (−0.62 **, −0.68 **, −0.62 **, −0.32 *), and Thermomicrobia (−0.28 *, −0.32 *, −0.29 *, −0.52 *).

#### Table 1. Correlation between soil nutrition and bacterial phyla.

| SOM      | TN     | AN     | TP     | AP     | TK     | AK     |
|----------|--------|--------|--------|--------|--------|--------|
| Cyanobacteria | 0.33   | −0.25  | −0.15  | 0.28   | −0.22  | −0.43  | −1.06  |
| Gemmatimonadetes | 0.12   | −0.26 *| 0.25   | 0.32   | −0.31 *| −0.19 *| −0.72 **|
| Actinobacteria  | 0.46 * | −0.86 **| 0.43 * | 0.32 * | −0.79 **| −0.86 **| −0.39 *|
| Acidobacteria  | 0.18   | −0.86 **| −0.34  | −0.11 *| −0.39 **| −0.79 **| −0.86 **|
| Chloroflexi    | 0.07   | −0.57 *| 0.17   | −0.31 *| −0.33 *| −0.49 *| −0.41 *|
| Verrucomicrobia| −0.04  | −0.62 **| 0.27   | −0.61 **| −0.32 *| −0.68 **| −0.62 **|
| Thermomicrobia | 0.14   | −0.28 *| 0.34   | −0.31 *| −0.52 *| −0.32 *| −0.29 *|
| Firmicutes     | 0.37 * | −0.35  | 0.47 * | 0.54 **| −0.54  | −0.13  | 0.25   |
| Proteobacteria | 0.56 * | −0.38  | 0.38 * | 0.25 * | −0.15  | −0.54  | −0.58  |
| Bacteroidetes  | 0.27 * | 0.26   | 0.67 **| 0.31 * | 0.38   | 0.40   | 0.15   |

* Indicates significant correlation at $P < 0.05$ level. ** Indicates significant correlation at $P < 0.01$ level.

The correlation coefficients between environmental variables and the top 10 bacterial classes under different systems are shown in Table 2. SOM was significantly positively correlated with Clostridia (0.63 **) and Bacilli (0.72 **). TN content was positively correlated with Clostridia (0.64 **), Bacilli (0.66 **) and Erpsipelotrichia (0.64 **), but negatively correlated with Alphaproteobacteria (−0.64 **) and Ktedonobacteria (−0.44 **). AN content was significantly positively correlated with Clostridia (0.71 **), Bacilli (0.82 **) and Erpsipelotrichia (0.50 **), while significantly negatively correlated with Alphaproteobacteria (−0.51 **) and Ktedonobacteria (−0.25 **). However, TP content had no significant correlation with soil microbes on class level. AP content was positively correlated with Clostridia (0.74 **) and Bacilli (0.76 *), whereas negatively correlated with Alphaproteobacteria (−0.59 **) and Ktedonobacteria (−0.29 **). TK content was positively correlated with Clostridia (0.54 **),
Actinobacteria (0.51 *), Bacilli (0.61 *) and Erysipelotrichia (0.60 *), but negatively correlated with Alphaproteobacteria (−0.64 **), Ktedonobacteria (−0.49 **). In addition, AK content was significantly negatively correlated with Gammaproteobacteria (−0.79 **).

Table 2. Correlation between soil nutrition and bacterial class.

| Class             | OM   | TN    | AN    | TP    | AP    | TK    | AK    |
|-------------------|------|-------|-------|-------|-------|-------|-------|
| Gammaproteobacteria | 0.10 | −0.19 | −0.11 | 0.21  | −0.09 | −0.32 | −0.79 **|
| Clostridia        | 0.63 ** | 0.64 ** | 0.71 ** | 0.24  | 0.74 ** | 0.54 * | 0.01  |
| Alphaproteobacteria | −0.19 | −0.64 ** | −0.51 * | 0.30  | −0.59 * | −0.64 ** | −0.29  |
| Ktedonobacteria   | −0.002 | −0.44 ** | −0.25 ** | −0.01 | −0.29 ** | −0.49 ** | −0.16  |
| Bacteroidia       | 0.36  | 0.80  | 0.65  | −0.01 | 0.77  | 0.74  | 0.08  |
| Actinobacteria    | −0.21 | 0.46  | 0.20  | −0.45 | 0.24  | 0.51 * | 0.46  |
| Bacilli           | 0.72 ** | 0.66 ** | 0.82 ** | 0.23  | 0.76 ** | 0.61 * | 0.14  |
| Acidimicrobia     | −0.39 | −0.26 | −0.35 | −0.18 | −0.40 | −0.10 | 0.19  |
| Acidobacteria     | 0.33  | −0.28 | −0.06 | 0.19  | −0.11 | −0.40 | −0.43 |
| Erysipelotrichia  | 0.36  | 0.64 ** | 0.50 * | −0.01 | 0.58 * | 0.60 * | 0.11  |

* Indicates significant correlation at P < 0.05 level. ** Indicates significant correlation at P < 0.01 level.

4. Discussion

In line with previous studies, Costa et al. [47] found that soil fertility improved in hedgerow intercropping systems involving six different hedgerow species, except for phosphorus. Similarly, Wen et al. [48] indicated that the soil nutrient content of the three intercropping systems (loquat-tea, waxberry-tea, and citrus-tea) was higher than that of the tea monoculture system. Intercropping management practices have been proven to influence the composition of soil nutrients [49], the results of the present study show that intercropping systems significantly increased the TN, TK, AN, AP, and SOM (Figure 2). The soil in the intercropping system is complex both temporarily and spatially as it contains microbiological, plant, physical and chemical properties [50]. The combination of different plant species, plant residues and vertical structure all affect the composition of the soil nutrients [51–53]. Plant diversity, in particular, had a significantly higher impact on soil nutrients than any other factor, which is likely due to the more developed roots of the OMT (tree-tea-tree) intercropping system and the surrounding soil and results in more favorable nutrients for root development. Therefore, the results presented in this work show that the OMT system is more suitable than the OT and MT systems for increasing soil nutrients (Figure 2).

Soil bacteria are an important part of the soil ecosystem, playing a crucial role in the cycling of nutrients and energy flow [7]. Numerous studies have indicated that soil microbes are often more diverse and abundant under intercropping than under monoculture systems in various soils [2,16,24]. The results of this study showed that intercropping system significantly increased the abundance of the soil bacterial community in the rhizosphere soil of tea (Figure 3). In general, the phylum bacteria Proteobacteria, Firmicutes, Actinobacteria accounted for the largest proportion in all three intercropping systems, implying that these phyla may play an important role in the tea-tree rhizosphere. In a previous study, it has been shown that the phylum Proteobacteria, Actinobacteria and Acidobacteria were highly responsive to N input [54]. Firmicutes are involved in the recycling and decomposition of SOM and are also consistently associated with disease suppression [55,56]. The relative abundance of Proteobacteria, Actinobacteria and Firmicutes was increased with N fertilization [54,57]. This might also explain the significant increase of TN and AN under the intercropping systems. Thus, it was speculated that the intercropping systems may increase the soil nutrient conversion cycle and might help the suppression of tea bushes disease.

In line with previous studies, Zhao et al. [58] found that Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes emerged as the dominant bacterial phyla in soil salinization samples. Similarly, Lin et al. [36] reported that the use of organic fertilizer significantly
increased in the relative abundance of Acidobacteriales and Gemmatimonadales. In this study, the abundances of Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes were positively correlated with the contents of AN, SOM and TP. However, Davis et al. [59] showed that Chloroflexi and Acidobacteria were slow-growing bacteria that generally prefer an oligotrophic environment. Chloroflexi have primarily been associated with extreme habitats and hypersaline environments [60,61]. The present results also showed the negative correlation between the content of soil nutrients (TN, TK, AK and AP) and the abundance of Chloroflexi and Acidobacteria. Therefore, it is suggested that the decreased Chloroflexi and Acidobacteria, and increased Proteobacteria and Firmicutes in the three intercropping systems were related to the improved soil nutrients (Figure 2).

Furthermore, on the class level, Bacteroidia, Erysipelotrichia, Clostridia, Actinobacteria and Bacilli were increased through positive interactions in the tea-tree systems, compared to the C system (Figure 3). The class Bacteroidia, which was found to be significantly enriched in soil of the tea-tree system, can metabolize complex SOM [62]. Similarly, Clostridia and Erysipelotrichia are involved in the decomposition of SOM [63]. Moreover, Clostridia play an important role in the decomposition of plant residues, which help to regulate the C cycle [64]. Actinobacteria are widely distributed in soil, where they play a crucial role in decomposition and humus formation [65]. The Bacillus species is one the most dominant rhizospheric bacterial/rhizobacteria species, which can help enhance plant growth and development by different mechanisms [66]. In addition, the Bacillus species plays an important role in N fixation and phosphorus, potassium, zinc, and iron cycles in soil [67,68]. Pandey and Palni [69] reported that Bacillus subtilis and B. mycoides are the dominant bacteria of the rhizosphere of tea bushes. The correlation results of this study indicate that Clostridia, Erysipelotrichia and Bacillus were significantly positively related with the soil nutrients (Table 2). Therefore, these results suggest that intercropping as a practice is a potential strategy for soil microbe diversity intensification of tea agriculture, as it can strengthen the soil nutrient conversion cycle. The increased soil nutrient availability due to intercropping systems were found to be the key factors associated with soil microbes [70,71]. Overall, these results suggest that the changes in bacterial composition would be induced in these intercropping system soils as plant diversity increases. The OT, MT, and OMT systems can increase soil microbe diversity compared to the C system (i.e., a monoculture) for tea cultivation, while the OMT system is the most suitable system.

5. Conclusions

An agroforestry-tea intercropping system improves soil nutrients status and enhances the abundance of soil microbial structure above those of a monoculture system. The increased plant diversity of the OMT system was better than the OT and MT systems for tea cultivation. Moreover, soil nutrients were highly correlated with soil microbial class, which was likely due to their positive impact on the tree-tea root system. The higher abundances of soil microbial classes under intercropping systems most likely resulted from shading and changes in root diversity. Therefore, these results highlight that intercropping of two or more tree species with tea improves resource availability and soil microbial class abundances. Future management should be cognizant of these agroforestry-tea intercropping systems, as they have multiple benefits over monoculture equivalents.

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