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Full Length Article

Comparative Study on Soil Microbial Community Structure and Diversity in Five Tea (*Camellia sinensis*) Cultivars

Songsong Gu[1], Qiulong Hu[1], Zhonghua Liu[1], Wenjun Xiao[1], Zhihua Gong[1], Yuqi Cheng[1], Ye Deng[2], Kai Feng[2] and Lin Tan[2*]
[1]*Hunan Agricultural University, Changsha 410128, Hunan Province, China*
[2]*Key Laboratory for Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (CAS), Beijing100085, China*
[*]For correspondence: hqltanlin@163.com; Fax: 86073184673707; abs@academicconf.com

Abstract

The soil microbial community associates with plant cultivar. Soil microbiomes can largely contribute to plant growth, health and agricultural production. However, the soil microbial community structure and diversity in relation to different tea (*Camellia sinensis*) cultivars remains unknown. By utilizing high-throughput 16S rRNA gene Illumina sequencing, we systematically studied and compared the microbial community and potential function in the bulk soils of 5 tea cultivars cultivated in a tea plantation for 15 years in central south of China. The tea cultivars were Bixiangzao (BXZ), Zaofengchun (ZFC), Rougui (RG), Maoxie (MX) and Baihaozao (BHZ). There were significant differences in soils communities for the relative abundances of six dominant phyla *Actinobacteria*, *candidate division WPS-2*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria* and *Thaumarchaeota* between both of Bixiangzao and Baihaozao and the other three cultivars ($p < 0.05$). Relative abundance of genus *Nitrososphaera* in soil of cultivar Bixiangzao was significantly higher than the soils of others cultivars. According to Chao1 and Richness diversity indices, the cultivar Bixiangzao had the highest soil microbial diversity relative to other cultivars. The principal coordinates analysis (PCoA) of soil microbial communities based on weighted Unifrac distances revealed a clear separation between Bixiangzao and other cultivars. Pearson relationship analysis showed that pH and TOC contents were major soil factors to positively influence the microbial diversity of different tea cultivars. Functional Annotation of Prokaryotic Taxa was adopted to predict that the percent relative abundances of aerobic ammonia oxidation, nitrification and fermentation functional bacteria in the soils of cultivar Bixiangzao were significantly higher compared with other cultivars. Our results indicated that the selection of cultivar Bixiangzao may support and foster a large number of specific microbiota and a favorable microecological environment in tea plantation. This study provided theoretical basis for future studies on rhizosphere effect guiding the tea plantation soil community influenced by different tea cultivars.

Keyword: Tea cultivars; Microbial diversity; Community structure; Functional prediction

Introduction

Tea (*Camellia sinensis*) is an evergreen perennial plant found mainly in tropical and subtropical countries, including China, India, Pakistan, Kenya, etc. The areas of tea cultivation in China have been expanding due to its high economic value and had covered 3,680 hectares by 2017, accounting for about 60% of the world's total tea area and over 40% of the world's total tea production. However, as more countries establish tea plantations and the general expansion of tea cultivation areas, the competitive advantage of China's tea industry in the world is declining, mainly due to the overall quality and the single yield of tea being low (Yan *et al*., 2018). The single yield reductions and decline in soil quality have become a major problem for sustainable tea production (Han *et al*., 2007).

Soil microbiomes can largely contribute to plant growth, health and agricultural production (Mendes *et al*., 2013). Previous studies showed that soil microorganisms played an essential role in soil fertility, plant stress resistance, growth and development and productivity (Eisenhauer *et al*., 2012). The nitrogen-fixing bacteria such as *Rhizobium* and *Frankia* could increase crop yield and enhance nitrogen uptake of plants (Adesemoye and Kloepper, 2009). *Pseudomonas*, *Bacillus* and *Agrobacterium* could promote plant growth by producing phytohormone or molecular signal, competing growth sites, ferrum carriers and other pro-growth mechanisms and enhancing resistance to pathogens (Richardson *et al*., 2009; Jetiyanonal and Plianbangchang, 2012).

There appears to be intricate relationship between plant cultivar and soil microbiome that are associated with promoting the plant growth and participating in the nutrient cycle in agro-ecosystems (Pathan *et al*., 2015). Different plant cultivars possessed specific phenotypic characteristics, such as root properties (Czarnota *et al*., 2003;

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Bais *et al*., 2006), which were key factors to influence microbial assembly and community composition (Grayston and Colin, 1996; Baudoin *et al*., 2003; Haichar *et al*., 2008). Plants could regulate and influence the biomass, species and ecological distribution of soil microorganisms through root exudates (Mansouri *et al*., 2002). Direct selection of plant genotypes for Promoting beneficial rhizobacteria might confer protection against pathogens and the abiotic stress resistance to plants (Schlemper *et al*., 2017). The plant-microbial interaction could improve the growth of plant through phytohormone production which can defense against phytopathogens through competition, antagonism and hyperparasitism and withstand extreme heat and other abiotic stresses (Dong *et al*., 2018). Therefore, characterization of soil microbial community of different tea cultivars would contribute to further development of rhizosphere effect for promoting plant growth and improving the yield and quality of tea plantation (Ramu *et al*., 2013).

In recent years, studies on the soil microbial diversity and community of plants using bioengineering technology at the molecular level have been extensively reported, but most objects of studies were focused on crop and forest (Gunina and Kuzyakov, 2015; Ren *et al*., 2018). Few studies focused on the microbial diversity and community in soils of different cultivars within the same species. Our study was designed to determine whether there were differences among microbial community diversity and structure in soils with the same spatial position under similar long terms agriculture management practices for five tea cultivars that are widely cultivated in different regions of south China. The objectives of this work were to (i) demonstrate the differences of soil microbial diversity and community structure for different tea cultivars; (ii) Show the differences of soil microbial community composition for different tea cultivars. (iii) analysis the correlation between soil properties and microbial communities of different tea cultivar; (iiii) predict the potential function of microbial community in soils of different tea cultivars.

Material and Methods

Site and Sample Collection

Different tea cultivars were all cultivated in a tea plantation of Changsha city (28°11′ N, 112°59′ E), Hunan Province, southeastern China in 2003. The tea cultivars were Bixiangzao (BXZ), Zaofengchun (ZFC), Rougui (RG), Maoxie (MX) and Baihaozao (BHZ). Five tea cultivars were cultivated in respective experimental plot with size of larger than 667 m^2 in a tea plantation. The experimental plots were separated by a 1 m wide walkway. The agriculture management practices for five tea cultivars was the same since 2003. Soil samples were taken from tea plantation at the soil depths of 0-20 cm. A total of 25 soil cores were taken from each experimental plot of tea cultivars by using the checkerboard sampling method in August 2017. Five soil cores were mixed to form a composite sample, and a total of 25 soil samples were collected and stored in the freezer at -80°C before DNA-extraction. The soil samples were divided into two parts, one part was assigned for detecting the physicochemical properties while the other part was assigned for performing molecular analysis.

Physicochemical Characteristics of Soils

The following physicochemical properties of collected soil samples were determined as follows: pH, total organic carbon (TOC), nitrate nitrogen (NO_3-N) and ammonia nitrogen (NH_4-N), total nitrogen (TN), available phosphorus (AP), moisture, total phosphorus (TP) (Viji and Shrinithivihahshini, 2017). Soil analyses were performed by the Laboratory of Soil Analyses of the Department of Nanjing Institute of Geography, CAS, using standard methods (University, 2006).

DNA Extraction and Amplicon Sequencing

Total DNA was extracted from 0.5 g soil samples with the Fast DNA Spin kit (MP Biomedicals LLC, USA). DNA quality and concentration were detected by a NanoDrop Spectrophotometer (Nano-100, Aosheng Instrument Co Ltd.). The extracted DNA was used as a template for amplifying the V4 region of the 16S rRNA gene by using the 515F (5'-GTGCCAGCMGCCGCGGTAA-3)/806R (5'-GGACTACHVGGGTWTCTAAT-3') primers. PCR (polymerase chain reaction) amplification was performed in a 50 μL reaction system containing 1 μL of template DNA (20 - 30 ng/μL), 1.5 μL of both forward and reverse primers (10 μM), 0.5 μL Taq DNA Enzyme, 5 μL 10 × PCR buffer, 1.5 μL dNTP mixture and 39 μL ddH_2O. The thermal cycle conditions were as follows: initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 20 s, 57°C for 25 s and 68°C for 45 s, subsequently ending at 68°C with a final extension step of 10 min and finally keep systems at 4°C before purification on SelectCycler II (Select BioProduct).

The PCR products were purified by E.Z.N.A.™ Gel Extraction Kit (Omega Bio-tek, Norcross, GA, USA). The purified amplicons were quantified with a Qubit fluorometer (Life technologies Holdings Pte Ltd, Singapore) and constructed the library with VAHTSTM Nano DNA Library Prep Kit for Illumina® (Vazyme Biotech Co., Ltd, Nanjing, China). The samples were sequenced by Miseq sequencing machine (Illumina) at Central South University, China.

Sequence Preprocessing and Bioinformatics Approaches

The barcodes were used to assign the raw reads to samples (with one mismatch allowed). After removal of barcodes and primers, pair-ended sequences were quality-filtered by using Flash program (Mago and Salzberg, 2011). UPARSE algorithm (Edgar, 2013) was used to remove chimeras and generate OTU (operational taxonomic units) table at a 97%

similarity level without any singletons being discarded. All the sequence preprocessing was performed in an in-house pipeline (http://mem.rcees.ac.cn:8080) with series of bioinformatics tools.

Ecological and Statistical Analysis

Two kinds of alpha-diversity Chao1 values (Chao, 1984) and Richness were calculated to assess the diversity of soils microbial community associated with different tea cultivars. Unweighted principal coordinate analysis (PCoA) based on UniFrac matrix was performed for evaluating the microbial community structure difference (Lozupone and Knight, 2005). The Pearson correlation approaches were applied to correlate alpha-diversity and physicochemical characteristics of soils (PCC: Pearson Correlation Coefficient). The microbial ecological function profiles were predicted by FAPROTAX (Functional Annotation of Prokaryotic Taxa; (Louca and Doebeli, 2016). The significant differences between each two groups and among multiple groups were determined by two-tailed Student's t-test and one-way analysis of variances (ANOVA), respectively.

Result

The Soil Microbial Community Composition Associated with Different Tea Cultivars

A total number of 311, 981 valid reads and 9, 476 OTUs were obtained from the 25 soil samples by using a high-throughput sequencing analysis. Rarefaction curves (Fig. S1) tended to approach a saturation plateau indicating that the sequencing depths were sufficient for downstream analysis. All the OTUs were assigned to 44 different phyla (Fig. S2). Majority of sequences (84.23%) of five cultivars soils were identified as seven phylum *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Thaumarchaeota*, *Firmicutes* and *Verrucomicrobia*. In addition, the low abundances of phylum *Planctomycetes*, *Bacteroidetes* and *candidate division WPS-2* were presented in all samples (with relative abundance of 1.4% - 2%).

Relative abundances of dominant phyla within soil microbial communities of different cultivars are shown in Table 1. There were significant differences between both of BXZ, BHZ and the other three cultivars ($P < 0.05$) for the relative abundances of six dominant phyla, including *Actinobacteria*, *candidate division WPS-2*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria* and *Thaumarchaeota*. The relative abundance of most dominant phylum *Proteobacteria* exhibited significant differences between cultivar BHZ (37.98%) and others cultivars (less than 21.63%), however the lowest abundance of phylum *candidate division WPS-2* (0.17%) was observed in soil of cultivar BHZ than soils of other cultivars (1.26% < relative abundance < 2.20%). Additionally, the soil of cultivar BHZ had a significantly lower abundance of *Chloroflexi* (5.10%) than other cultivars

Table 1: Relative abundances of dominant phyla within soil microbial communities of different tea cultivars

Domain phyla	Different cultivars and Relative abundances (%)				
	BXZ	ZFC	RG	MX	BHZ
Acidobacteria	22.90a	26.75a	25.93a	19.20a	21.84a
Actinobacteria	6.49b	9.01ab	7.66b	8.77ab	12.65a
Bacteroidetes	2.10a	0.98a	1.38a	1.30a	2.52a
candidate division WPS-2	1.53a	2.04a	1.26a	2.20a	0.17b
Chloroflexi	10.78ab	16.72a	16.12a	20.06a	5.10b
Firmicutes	4.76a	6.74a	4.99a	6.94a	3.41a
Planctomycetes	1.12b	3.63a	1.71b	2.01b	0.69b
Proteobacteria	21.13b	18.60b	21.63b	19.84b	37.98a
Thaumarchaeota	15.43a	2.05b	2.52b	2.69b	2.03b
Verrucomicrobia	1.46a	2.40a	2.96a	2.76a	1.90a

BXZ (*cv.Bixiangzao*), ZFC (*cv. Zaofengchun*), RG (*cv.Rougui*), MX (*cv.Maoxie*) and BHZ (*cv. Baihaozao*), similarly hereinafter

(10.78% < relative abundance < 20.02%). the soil of cultivar BHZ had the highest abundance (15.43%) of *Thaumarchaeota* than others cultivar (less than 2.69%) and a lower abundance (6.49%) of *Actinobacteria* than cultivar ZFC (9.01%) and MX (8.77%) while the soil of cultivar BHZ (12.65%) had the highest abundance. In addition, the soil of cultivar ZFC had the most abundance of *Planctomycetes* than others cultivars ($P < 0.05$).

To provide deeper understanding of the effects of tea cultivars on soil microbiomes, the dominant genera were analyzed (Fig. 1a). A total of 434 genera were detected at the genus level. And the relative abundances (> 1%) of the top 10 identified microbial genera exhibited significant differences among the soils of five tea cultivars. The soil of cultivar BXZ had the highest abundance (43.04%) of the top 10 dominant genera, while cultivar BHZ had the lowest abundance (28.43%). Additionally, the soil of cultivar BXZ possessed the highest abundance of *Nitrososphaera*, which was the fourth dominant genus observed in BHZ, RG, MX and ZFC. In order to investigate the similarities and differences among these composition of soil microbial communities of five tea cultivars, the shared and specific species was analyzed by using the Venn diagram (Fig. 1b). The results exhibited that the shared species among the soils of five cultivars accounted for different percentages of each own microbial community, 28.80%, 33.65%, 38.37%, 44.36% and 40% for BXZ, BHZ, RG, MX and ZFC cultivars, respectively. The percentage of specific species for microbial communities in soils of five cultivars were follow as: 32.34%, 27.13%, 13.63% 17.62% and 13.49% for BXZ, BHZ, RG, MX and ZFC cultivars, respectively. The soil of cultivar BXZ had the highest proportion of specific species. All the above results reveled that soil microbial community composition was significant different in soils of different cultivars.

The Soil Microbial Community Diversity and Structure of Different Tea Cultivars

According to Chao1 (Fig. 2a) and Richness (Fig. 2b) diversity indices, the soil of cultivar BXZ had the highest alpha-diversity than other tea cultivars while the soils of MX

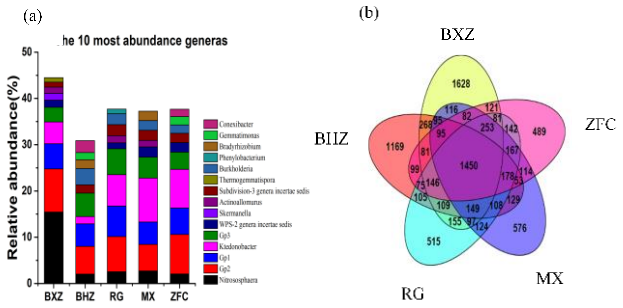

Fig. 1: OTU analyses of the different tea cultivar libraries. (a) The relative abundances of the domain phyla in five tea cultivar soils at the phylum level; (b) The venn diagram showing the unique and shared OTUs (3% distance level). BXZ (C.sinensis cv.Bixiangzao), ZFC (C.sinensis cv. Zaofengchun), RG (C.sinensis cv.Rougui), MX (C.sinensis cv.Maoxie) and BHZ (C.sinensis cv. Baihaozao), similarly hereinafter

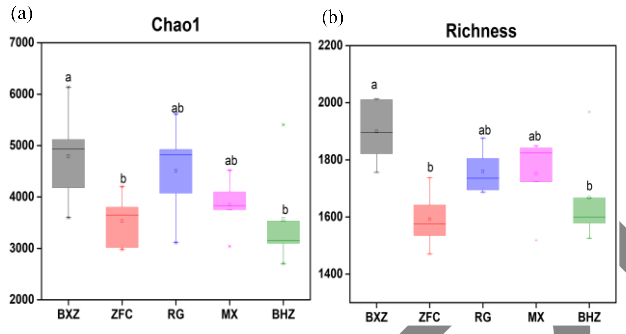

Fig. 2: The Chao1 (a) and Richness (b) diversity indices for microbial 16S rRNA genes of different tea cultivar soils. Different letters indicate statistically significant differences (*$P < 0.05$)

and BHZ cultivars had the lowest diversity. The heatmap analysis was conducted at the genus level for all dominant genera (The relative abundances > 1%) from all 25 soil samples of five tea cultivars. The results indicated that ZFC, RG and MX cultivars clustered together, whereas the soils of BHZ and BXZ cultivars were separated (Fig. 3). Moreover, soil microbial community of cultivar ZFC was found to be similar to the cultivar RG. The principal coordinates analysis (PCoA) of soil microbial communities using weighted UniFrac distances based on the OTU distribution across samples revealed a clear separation between BXZ and other cultivars, and also showed the partial overlaps among ZFC, RG, MX and BHZ cultivars in spatial distribution (Fig. 4). Furthermore, the significant differences in soil microbial communities between BXZ and other four cultivars were also supported by the results of dissimilarity test using the MRPP, ADONIS and PERMANOVA algorithms (Table S1).

The Correlation between Soil Properties and Microbial Community Diversity of Different Tea Cultivar Soils

The Pearson correlation approach based on both Jaccard and Bray-Curtis distance was performed to assess the relationship

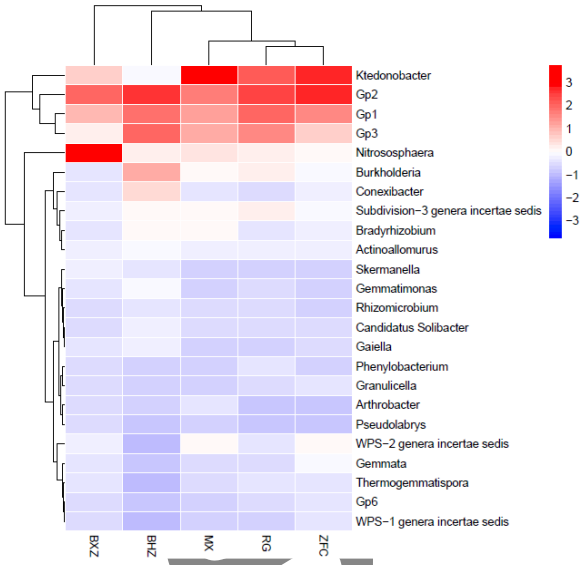

Fig. 3: Hierarchically clustered heatmap of the microbial genera detected across all samples. The microbial phylogenetic tree was calculated by using neighbour-joining method and the relationship among samples was determined by Bray-Curtis distance and complete clustering method. The relative values for microbial genus are indicated by color intensity with the legend at the right of the figure

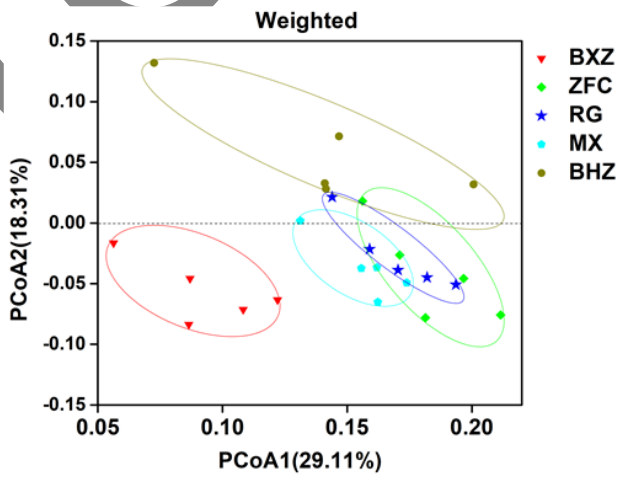

Fig. 4: The PCoA plot of microbial community structure and composition in five tea cultivar soils

between soil properties and the diversity of microbial community diversity of five cultivars soils (Table 2). The results indicated that the soil moisture had a significant negative correlation with Shannon (PCC= -0.458, *P*= 0.019), Inv-Simpson (PCC= -0.577, *P*= 0.002) and Pielou-Evenness (PCC= -0.486, *P*= 0.012) indices, while had a significant positive correlation with Chao1 (PCC= 0.391, *P*= 0.048) and PD (PCC=0.396, *P*= 0.045) indices; pH value showed significant positive correlation with Shannon (PCC=0.396, *P*= 0.045) and Inv-Simpson (PCC= 0.436, *P*= 0.026) indices; AP showed a significant negative correlation with Inv-Simpson (PCC= -0.391, *P*= 0.048) index;

Table 2: The correlation between soil properties and microbial diversity based on Pearson analysis approach

Soil properties	Shannon		Inv-Simpson		Richness		Pielou-Evenness		Chao1		PD	
	PCC	*P*	PCC	*P*	PCC	*P*	PCC	*P*	PCC	*P*	PCC	*P*
TP (mg kg^{-1})	-0.312	0.121	-0.370	0.062	-0.133	0.518	-0.135	0.509	-0.011	0.958	0.089	0.667
TN (mg kg^{-1})	-0.114	0.581	0.129	0.531	-0.240	0.237	0.153	0.456	-0.389*	0.050	-0.470*	0.015
NO_3N (mg kg^{-1})	-0.079	0.703	-0.010	0.961	-0.154	0.452	0.082	0.692	-0.198	0.332	-0.259	0.201
NH_4N (mg kg^{-1})	0.011	0.957	0.137	0.503	-0.067	0.746	0.070	0.735	-0.173	0.398	-0.369	0.064
AP (mg kg^{-1})	-0.349	0.080	-0.391*	0.048	-0.040	0.845	-0.274	0.176	0.010	0.963	-0.029	0.890
TOC (%)	-0.095	0.643	0.112	0.586	-0.194	0.342	0.098	0.636	-0.352	0.078	-0.603**	0.001
Moisture (%)	-0.458*	0.019	-0.577**	0.002	0.114	0.578	-0.486*	0.012	0.391*	0.048	0.396*	0.045
pH	0.396*	0.045	0.436*	0.026	0.023	0.911	0.311	0.122	-0.089	0.666	0.102	0.619

PCC= Pearson correlation coefficient

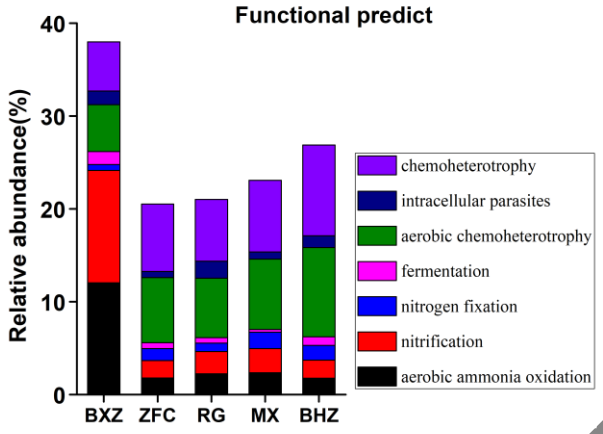

Fig. 5: Function predictions of microbial communities in five tea cultivar soils by FAPROTAX

TN showed a significant negative correlation with Chao1(PCC= -0.389, *P*= 0.05) and PD (PCC= -0.47, *P*= 0.015) indices; TOC showed a significant negative correlation with PD (PCC= -0.603, *P*= 0.001) index. Therefore, pH value and TOC content were the key physicochemical factors to positively influence the alpha-diversity of soil communities of different tea cultivars.

Functional Prediction of Microbial Communities of Different Tea Cultivars Soils

Functional Annotation of Prokaryotic Taxa (FAPROTAX (Louca *et al*., 2016)) was conducted to predict micro-ecology functions to the OTUs for bacteria in different tea cultivar soils (Fig. 5). We loaded 90 functional groups derived form 7,700 members (4,668 unique members). The dominant functional groups (> 1%) were belonged to aerobic ammonia oxidation, nitrification, nitrogen fixation, fermentation, aerobic chemoheterotrophy, chemoheterotrophy, intracellular parasites. The correlations between microbial classes and functional groups indicated that most microbial classes possessed the functions of fermentation, aerobic chemoheterotrophy, and hemoheterotrophy with no significant different, and the proportion of aerobic ammonia oxidation, nitrification and fermentation functional bacteria in the soils of cultivar BXZ were significantly higher than others cultivars (Fig. 5).

Discussion

In this study, there were significant differences for microbial communities and physicochemical properties among soils samples collected from five tea cultivars, which indicated that different tea cultivar could shape specific soil microbial communities following the changes of soil properties after long-term growth with the same spatial position under the similar agriculture management practices. Previous research suggested that plant cultivars, soil properties and different management practices are the main factors to impact soil microbial community (Griffiths *et al*., 2011). Soil properties indirectly affect the diversity of soil microorganisms by altering their population and species (Barberán *et al*., 2012; East, 2013). The microbial community in rhizosphere soil is recruited from the main reservoir of resident microorganisms present in bulk soils (Li *et al*., 2016). These opinions implied that the rhizosphere effect of tea cultivar was the critical factor to determine microbial community diversities. In addition, soil microbial diversity and community play the essential roles in soil quality and ecological functions (Kennedy and Smith, 1995). Therefore, the selection of tea cultivars for improving the soil properties and fostering the favorable microecological environment is of great significance for the sustainable development of tea industry.

The composition analysis results showed that the relative abundances of dominant microorganisms in the five tea cultivar soils were significantly different at phylum and genus levels (Fig. 1). *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were the most dominant phyla in the all tea cultivars soils, which is supported by the previous study that *Acidobacteria* and *Proteobacteria* are dominant soil microbial taxa in tea plantation systems (Zhao *et al*., 2012). However, the soil of cultivar BHZ had the highest relative abundance of *Proteobacteria* (p <0.05) (Table 1), which was associated with large amounts of available nutrients (Li *et al*., 2016). In our study, BHZ cultivar soil possessed higher soil nutrition contents of TN, AP, TOC and TP than other cultivars through plant root-soil interactions under long term cultivation with same management practices (Table S2). This could be explained by the fact that the BHZ cultivar soil had the highest relative abundances of *Proteobacteria*.

Plant cultivar can influence the shaping of soil microbial community structure and function (Berg and Smalla, 2009). Waid (1999) pointed out that plant cultivar may be important factor to influence soil microbial community diversities. In the study by Pfeiffer *et al*. (2017), the stable key microbiome could be related to a similar pattern of plant exudates over plant growth stages, whereas dynamic core microbiome members may respond to the changes in root exudates over plant development. These studies highlight the possibility that selection of specific functional plant cultivars can contribute to plant growth, disease resistance, and bioremediation (Bell *et al*., 2014) for the soil ecological system. In addition, root exudate of teas contained appreciable quantities of oxalic acid, citric acid, malic acid (Nagata *et al*., 1993), which was proved to be an important factor that may lead to the soil acidification in tea plantations (Jayman and Sivasubramaniam, 1999). This might explain in part why the pH was the key soil factors to influence the microbial diversity of different tea cultivars. However, further work will be needed to confirm the effects of root exudates of teas on soil microbial communities.

The proportions of aerobic ammonia oxidation, nitrification and fermentation functional groups in the soil of cultivar BXZ was significantly higher than others cultivar (Fig. 5). Aerobic ammonia oxidation is thought to be driven by ammonia-oxidizing bacteria that play important roles in the process of nitrification and N cycling (Mundepi *et al*., 2017). Fermentative bacteria play essential roles in anaerobic degradation of organic matter in the soils (Glissmann and Conrad, 2000). The presence of above functional groups might be explained by the fact that a significant enrichment of the genus *Nitrososphaera* was observed in in soil of cultivar BXZ. *Nitrososphaera* is a genus of ammonia oxidizing archaeon in the Order *Nitrososphaerales* (Tournaa *et al*., 2011). Herlemann *et al*. (2013) found that the high nitrification activity in the soil is likely due to the fact that ammonia-oxidizing archaea can be primely adapted to soil oxygenation and/or plant root exudation (Herrmann *et al*., 2008). Soil pH greatly affect the abundance and diversity of ammonia-oxidizing archaea (Mundepi *et al*., 2017). In our results, lowest pH value was observed in the soil of cultivar BXZ. Therefore, we speculated that BXZ might be an acid-tolerant tea cultivar. Overall, the predicted functions of soil microbial communities of different tea cultivars could be helpful to select the favorable cultivar associated with particular microbial taxa for increasing tea productivity.

Conclusion

In this study, the long-term cultivation of different tea cultivars significantly impacted the soil properties, microbial diversity and community composition and structure. According to Chao1 and Richness diversity indices, BXZ soils possessed the highest level of microbial diversity compared with other cultivar samples. There were significant differences in soils community for the relative abundances of six dominant phyla *Actinobacteria*, *candidate division WPS-2*, *Chloroflexi*, *Planctomycetes*, *Proteobacteria* and *Thaumarchaeota* between both of BXZ, BHZ and the other three cultivars ($p < 0.05$). In addition, the relative abundance of genus *Nitrososphaera* in soils of BXZ cultivar was significantly higher than others cultivars. PCoA plots of soils communities revealed a clear separation between BXZ and other cultivars. The relationship analysis between soil properties and microbial diversity showed that pH and TOC content were key physicochemical factors to positively influence microbial diversity in soils of different tea cultivars. Percent relative abundances of aerobic ammonia oxidation, nitrification and fermentation functional bacteria in the soil of cultivar BXZ were predicted to be significantly higher compared with other cultivars. Finally, the selection of optimal tea cultivars for sustainable agricultural measures are important to improve soil microbial diversity, community and function microbiomes in tropical and subtropical China, which will be the focus of our future research.

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Reference

Adesemoye, A.O. and J.W. Kloepper, 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.*, 85: 1–12

Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy and J.M. Vivanco, 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, 57: 233–266

Barberán, A., S.T. Bates, E.O. Casamayor and N. Fierer, 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.*, 6: 343–351

Baudoin, E., E. Benizri and A. Guckert, 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol. Biochem.*, 35: 1183–1192

Bell, T.H., S.E.D. Hassan, A.L. Lauron-Moreau, F. Al-Otaibi, M. Hijri, E. Yergeau and M. St-Arnaud, 2014. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *ISME J.*, 8: 331–343

Berg, G. and K. Smalla, 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.*, 68: 1–13

Chao, A., 1984. Nonparametric Estimation of the Number of Classes in a Population. *Scand J. Stat.*, 11: 265–270

Czarnota, M.A., A.M. Rimando and L.A. Weston, 2003. Evaluation of root exudates of seven sorghum accessions. *J. Chem. Ecol.*, 29: 2073–2083

Dong, M., Z. Yang, G. Cheng, L. Peng, Q. Xu and J. Xu, 2018. Diversity of the bacterial microbiome in the roots of four saccharum species: s. Spontaneum, s. Robustum, s. Barberi, and s. Officinarum. *Frontiers Microbiol.*, 9: 267

East, R., 2013. Microbiom:soil science comes to life *Nature*, 501: S18–S19

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*, 10: 996

Eisenhauer, N., S. Scheu and A. Jousset, 2012. Bacterial diversity stabilizes community productivity. *PloS One*, 7, e34517

Glissmann, K. and R. Conrad, 2000. Fermentation pattern of methanogenic degradation of rice straw in anoxic paddy soil. *FEMS Microbiol. Ecol.*, 31: 117–126

Grayston, S.J. and C.D. Colin, 1996. Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (Larix eurolepis) and Sitka spruce (Picea sitchensis). *Tree Physiol.*, 16: 1031–1038

Griffiths, R.I., B.C. Thomson, P. James, T. Bell, M. Bailey and A.S. Whiteley, 2011. The bacterial biogeography of British soils. *Environ. Microbiol.*, 13: 1642–1654

Gunina, A. and Y. Kuzyakov, 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. *Soil Biol. Biochem.*, 90: 87–100

Haichar, F.E.Z., C. Marol, O. Berge, J.I. Rangel-Castro, J.I. Prosser, J.R.M. Balesdent, T. Heulin and W. Achouak, 2008. Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.*, 2: 1221–1230

Han, W., S.J. Kemmitt and P.C. Brookes, 2007. Soil microbial biomass and activity in Chinese tea gardens of varying stand age and productivity. *Soil Biol. Biochem.*, 39: 1468–1478

Herlemann, D.P.R., D. Lundin, M. Labrenz, K. Jürgens, Z. Zheng, H. Aspeborg and A.F. Andersson, 2013. Metagenomic de novo assembly of an aquatic representative of the verrucomicrobial class Spartobacteria. *mBio*, 4: e00569–00512

Herrmann, M., A.M. Saunders and A. Schramm, 2008. Archaea dominate the ammonia-oxidizing community in the rhizosphere of the freshwater macrophyte Littorella uniflora. *Appl. Environ. Microbiol.*, 74: 3279–3283

Jayman, T.C.Z. and S. Sivasubramaniam, 1999. Release of bound iron and aluminium from soils by the root exudates of tea (camellia sinensis) plants. *J. Sci. Fd Agric.*, 26: 1895–1898

Jetiyanonal, K. and P. Plianbangchang, 2012. Potential of Bacillus cereus strain RS87 for partial replacement of chemical fertilisers in the production of Thai rice cultivars. *J. Sci. Food Agric.*, 92: 1080–1085

Kennedy, A.C. and K.L. Smith, 1995. Soil microbial diversity and the sustainability of agriculture soils. *Plant Soil*, 170: 75–86

Li, Y.C., Z. Li, Z.W. Li, Y.H. Jiang, B.Q. Weng and W.X. Lin, 2016. Variations of rhizosphere bacterial communities in tea (Camellia sinensis L.) continuous cropping soil by high-throughput pyrosequencing approach. *J. Appl. Microbiol.*, 121: 787–799

Louca, S. and M. Doebeli, 2016. Transient dynamics of competitive exclusion in microbial communities. *Environ. Microbiol.*, 18: 1863–1874

Louca, S., L.W. Parfrey and M. Doebeli, 2016. Decoupling function and taxonomy in the global ocean microbiome. *Science*, 353: 1272–1277

Lozupone, C. and R. Knight, 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.*, 71: 8228–8235

Mago, T. and S.L. Salzberg, 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27: 2957–2963

Mansouri, H., A. Petit, P. Oger and Y. Dessaux, 2002. Engineered Rhizosphere: the Trophic Bias Generated by Opine-Producing Plants Is Independent of the Opine Type, the Soil Origin, and the Plant Species. *Appl. Environ. Microbiol.*, 68: 2562–2566

Mendes, R., P. Garbeva and J.M. Raaijmakers, 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic and human pathogenic microorganisms. *FEMS Microbiol. Rev.*, 37: 634–663

Mundepi, A., J. Norton, M. Cabrera, D. Franklin and M.Y. Habteselassie, 2017. Ammonia oxidizers in a grazing land with a history of poultry litter application. *J. Environ. Qual.*, 46: 994–1002

Nagata, T., M. Hayatsu and N. Kosuge, 1993. Aluminium kinetics in the tea plant using 27Al and 19F NMR. *Phytochemistry*, 32: 771–775

Pathan, S.I., M.T. Ceccherini, G. Pietramellara, M. Puschenreiter, L. Giagnoni, M. Arenella, Z. Varanini, P. Nannpier and G. Renella, 2015. Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. *Plant Soil*, 387: 413–424

Pfeiffer, S., B. Mitter, A. Oswald, B. Schloter-Hai, M. Schloter, S. Declerck and A. Sessitsch, 2017. Rhizosphere microbiomes of potato cultivated in the High Andes show stable and dynamic core microbiomes with different responses to plant development. *FEMS Microbiol. Ecol.*, 2

Ramu, P., C. Billot, J.F. Rami, S. Senthilvel, H.D. Upadhyaya, L.A. Reddy and C.T. Hash, 2013. Assessment of genetic diversity in the sorghum reference set using EST-SSR markers. *Theor. Appl. Genet.*, 126: 2051–2064

Ren, C., T. Wang, Y. Xu, J. Deng, F. Zhao, G. Yang, X. Han, Y. Feng and G. Ren, 2018. Differential soil microbial community responses to the linkage of soil organic carbon fractions with respiration across land-use changes. *For. Ecol. Manage.*, 409: 170–178

Richardson, A.E., J.M. Barea, A.M. McNeill and C. Prigent-Combaret, 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*, 321: 305–339

Schlemper, T.R., M.F.A. Leite, A.R. Lucheta, M. Shimels, H.J. Bouwmeester, J.A.V. Veen and E.E. Kuramae, 2017. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *FEMS Microbiol. Ecol.*, 93: 1–11

Tournaa, M., M. Stieglmeier, A. Spanga, M. Könneke, A. Schintlmeister, T. Urich, M. Engel, M. Schloter, M. Wagner, A. Richter and C. Schleper, 2011. Nitrososphaera viennensis, an ammonia oxidizing archaeon from soil. *Proc. Natl. Acad. Sci. USA*, 108: 8420–8425

University, A.S., 2006. Soil Properties Analysis

Viji, R. and N.D. Shrinithivihahshini, 2017. An assessment of water quality parameters and survival of indicator in pilgrimage place of Velankanni, Tamil Nadu, India. *Ocean Coastal Manage.*, 146: 36–42

Waid, J.S., 1999. Does soil biodiversity depend upon metabiotic activity and influences. *Appl. Soil. Ecol.*, 13: 151–158

Yan, P., C. Shen, L.C. Fan, X. Li, L.P. Zhang, L. Zhang and W.Y. Han, 2018. Tea planting affects soil acidification and nitrogen and phosphorus distribution in soil. *Agric. Ecosyst. Environ.*, 254: 20–25

Zhao, J., X. Wu, C. Nie, T. Wu, W. Dai, H. Liu and R. Yang, 2012. Analysis of unculturable bacterial communities in tea orchard soils based on nested PCR-DGGE. *World J. Microbiol. Biotechnol.*, 28: 1967–1979

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