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Studies on fungal communities and functional guilds shift in tea continuous cropping soils by high-throughput sequencing

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Abstract

Purpose: Although soil fungal communities are considered important in tea orchard ecosystems, experimental research on their responses to the long-term continuous ratooning cultivation of tea plants are limited.

Methods: In this study, Illumina high-throughput sequencing technology was employed to investigate soil fungal communities in tea orchards with continuous cropping histories of 0, 1, 10, and 20 years.

Results: Results indicated that the predominant phyla were Ascomycota and Basidiomycota in all tea soil samples. Plant pathogen fungi *Alternaria* was the most predominant genus in the 10- and 20-year soils and significantly higher than that in the 0- and 1-year soils. FUNGuild revealed that the symbiotrophs in the 1-year soil (8.00%) was markedly higher than those in the 0-, 10-, and 20-year soils (1.43%~2.47%). The saprotrophs in the 20-year soils was approximately two-fold higher than those identified in the 0-, 1-, and 10-year soils. The pathotroph–saprotroph–symbiotroph fungi were higher in the 10- and 20-year soils in comparison to the 0- and 1-year soils as expected. Diversity analysis showed that the indices of Shannon and Simpson in the 1-year soils were higher than those in other treatments. Redundancy analysis suggested that fungal community structure and function were evidently interrelated to pH and exchangeable aluminum in the soils, respectively.

Conclusion: In summary, the long-term continuous ratooning cultivation of tea plants changed the fungal communities in the rhizosphere, enriched saprotrophs and plant pathogens (*Alternaria* spp.), and reduced beneficial fungi (symbiotrophs). Results of this work can be used to explore reasonable management measures, such as microbial fertilizer application, and eventually relieve the long-term monoculture problems of tea plants.

Keywords: *Camellia sinensis*, Continuous cropping, Fungal community, Illumina sequencing, Rhizosphere

Introduction

Tea (*Camellia sinensis* L.), as a major economically valuable crop in China, its plantation area increased to 2.74 million ha in 2014, approximately accounting for 74.8% of the world (FAO 2014). However, soil degradation and yield decline frequently occur after long-term continuous ratoon cropping of tea plants, an event regarded as a continuous cropping obstacle. Presently, the worsening

of physicochemical properties, the accumulation of auto-toxic substances, and the imbalance of microbial flora are considered the main factors contributing to this phenomenon (Lin et al. 2007; Zhou and Wu 2012).

Previous studies have investigated the shifts of soil microbial communities in the tea continuous cropping system. Wang (2014) found that the abundance of bacteria and fungi obviously decreased with the increase in continuous cropping years of tea plants; moreover, Acidobacteria, Actinobacteria, Chloroflexi, and Ascomycota were more predominant in younger tea orchard soils, whereas Proteobacteria and Bacteroidetes were less abundant in the older ones. Some researchers revealed

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that long-term tea continuous cropping changed the bacterial community structure, reduced microbial diversity, and caused the decrease in potentially beneficial plant-associated bacteria in continuous cropping soils (Zhao et al. 2012; Li et al. 2016). As a substantial and diverse group of soil microorganisms, fungi consist of multiple functional groups; for instance, both decomposers, mutualists, and pathogens belong in fungi (Bardgett and Wardle, 2010). Several recent studies have paid close attention to the disruption of soil fungal communities in the continuous cropping cultivation of many crops. Zhou and Wu (2012) revealed that fungal pathogen populations, such as *Fusarium oxysporum*, easily accumulated after continuous cropping of cucumber and harmed plants. Li et al. (2014) reported that the continuous cropping of peanut clearly promoted fungal pathogen abundance at the cost of potential plant-beneficial fungi and perhaps resulted in the yield decline of consecutive peanut monoculture. However, knowledge regarding the response of the rhizospheric soil fungal community to the continuous ratoon cropping of tea plants is limited. Previous studies have found that the soil microbial community is affected by various environmental factors, such as pH, carbon availability, and soil moisture (Rousk et al. 2010; Grantina et al. 2011; Brockett et al. 2012). Therefore, we hypothesized that tea continuous ratoon cropping can generate the alterations in a soil fungal community. Moreover, this alteration would be regulated by the soil physiochemical properties, which could cause negative effects on tea growth and yield.

The high-throughput sequencing technology is frequently employed for the detection of the diversity of soil microbial community and their relative abundance and evolution (Acosta-Martínez et al. 2008; Han et al. 2016; Tan et al. 2017). Moreover, FUNGuild is a new approach and has been used to ascertain the ecological function information of a fungal community (Nguyen et al. 2016). 'Tie Guanyin', a tea cultivar that is one of 10 best known teas in China, is generally grown in the Anxi County of Fujian Province in China. This study aimed to determine the fungal community structures and function in rhizospheric soils of 'Tie Guanyin' tea orchards with 0, 1, 10, and 20 years of continuous cropping and investigate the possible environmental factors resulting in the variation in soil fungal communities. Results will provide theoretical and experimental evidence for the further study of the mechanisms and field management strategies of the continuous cropping obstacles of tea plants.

Materials and methods

Cultivation area descriptions

The sampling areas were situated in the tea orchards at Gande Town (25°18' N, 117°51' E) in Anxi County of

Fujian Province, China. The cultivation region is typically monsoonal with an annual average temperature of 15–18 °C and rainfall of approximately 1700–1900 mm. Tea orchards, which have been continuously cultivated for 1, 10, and 20 years, were selected. The adjacent wasteland sampled for comparison (referred to as "0 year") had no tea planting record and was grown with sparse grasses. Three landscape replications were set for each treatment. These tea orchards suffered the similar environmental conditions (e.g., slope position, slope aspect, and altitude) and comparable agronomic measures. A compound fertilizer (21% N, 12% P, and 12% K) was added in the soils of these orchards in June, August, and September of every year at the rate of 750 kg ha⁻¹ each time. The prunings of upper leaves and buds were left in tea orchards as surface mulch.

Yield investigation and chemical quality assay

'Tie Guanyin' tea plant is managed as a shrub through pruning operations. One-year-old tea orchards are in the young stand stage and scarcely harvested. Ten and 20-year-old tea orchards are in the mature stand stage, and top leaves (one bud with three young leaves) of each stem of tea plants are usually harvested twice a year. On 1 October 2015, six rows of tea trees were randomly chosen in each tea orchard, and one sampling point of 33 cm × 33 cm was set in each row. All sprouts (one bud with three young leaves) in each sampling sites were collected, and the weight was measured by external balance. The harvested sprouts were heated in an oven at 150 °C for the deactivation enzymes and then dried at 65 °C. The dry samples were crushed into powder and subsequently stored in a desiccator for chemical quality determination. Total amino acid, caffeine, tea polyphenol, and catechin contents were determined according to the methods of Zhang (2009).

Soil sampling and chemical properties determining

The soils were sampled from various orchards with different continuous ratoon cropping years (0, 1, 10, and 20 years). Each treatment included three sites, resulting in a total of 12 soil samples. Six tea trees were randomly selected at each site, and the rhizospheric soils collected from each tea tree were pooled to form a composite sample. Rhizospheric soils represent the soil zone at approximate 1–5 mm around the root. All collected soil samples were classified as oxisol in accordance with the Soil Survey Staff (2010) and were developed from the granite. The soil samples were collected on 1 October 2015 and taken to the lab in correctly labeled and sealed valve bags. After being screened by a 2 mm sieve, each soil sample was separated into two parts: one portion dried in air was used to determine chemical properties,

and the remainder was stored in ultra-low temperature freezer to extract DNA.

The pH of soil was measured in a 1:2.5 of soil-H₂O suspension through a glass electrode. Soil organic carbon (SOC) was ascertained through the dichromate oxidation method. Total nitrogen (TN) was measured by the Kjeldahl digestion procedure, total phosphorus (TP) was analyzed after fusion with sodium carbonate, and total potassium (TK) was identified using the NaOH melt flamer procedure (Bao 2000). Available N (AN), available phosphorus (AP), and available potassium (AK) were measured via the alkaline hydrolysable, molybdenum blue, and flame photometry methods, respectively. The exchangeable H⁺ and Al³⁺ (EH⁺ and EAl³⁺) of soil were extracted using 1.0 M KCl and then titrated to pH 7.0 using 0.25 M NaOH. Cation exchange capacity (CEC) was gauged through the ammonium acetate compulsory displacement method (Lu 2000).

Soil DNA extraction, PCR amplification, and Illumina sequencing

Soil genomic DNA was extracted by using the Biofast Soil Genomic DNA Extraction Kit (Hangzhou Bioer Technology Co., Ltd., Hangzhou in China) according to the manufacturer's instructions. Before PCR amplification, three successive DNA extractions of each soil sample were pooled to optimize DNA extraction deviation. DNA purity and concentration were assessed by the NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The internal transcribed spacer 2 (ITS2) was amplified by a specific pair of primers (ITS2F: GCATCGATGAAGAACGCAGC, ITS2R: TCC TCCGCTTATTGATATGC) with a barcode. The PCR reaction was carried out in 30 µL of reaction buffer with 15 µL Phusion® High-Fidelity PCR Master Mix solution (New England Biolabs, Waltham, MA), 0.2 µM forward and reverse primers, and 10 ng genomic DNA. The PCR reaction procedure included an initial denaturation at 98 °C for 1 min, then followed by 30 cycles of denaturizing at 98 °C for 10 s, annealing at 50 °C for 30 s, elongating at 72 °C for 30 s, and a subsequent extension at 72 °C for 5 min. PCR products were monitored on 2% agarose gel electrophoresis, and samples with a bright strip between about 400 and 450 bp were chosen for further experiments. PCR products were mixed in the equidensity and purified with a QIAquick Gel Extraction Kit solution (Qiagen, Hilden, Germany). The purified PCR products were used to sequence through the Illumina HiSeq2500 PE250 platforms in Novogen Bioinformatics Technology Co., Ltd., Beijing in China.

The reads obtained by sequencing were matched to each sample on the basis of its unique barcode and curtailed by omitting the sequences of barcode and primer. Paired-end reads were merged from the primary DNA

fragments by the FLASH software (V1.2.7) to obtain the raw tags. Quality filtering of the raw tags was implemented in accordance with the QIIME (V1.9.1) quality-controlled process. Chimera sequences were checked and deleted by the USEARCH software on account of the UCHIME algorithm.

Statistical and bioinformatics analysis

The sequence reads with ≥ 97% similarity was designated to the same operational taxonomic units (OTUs) through the UPARSE software (UPARSE v7.0.1001). Species annotation was conducted through the UNITE Database (<https://unite.ut.ee/>) by using the BLAST algorithm, which was reckoned using the QIIME software (Version 1.9.1). The relative abundance of a given taxonomic group was calculated by the number of sequences belonged to this taxonomic group divided by the sum of all sequences per sample.

Alpha diversity was adopted to analyze the complexity of species diversity of sample according to six indices, i.e., the observed species, Shannon, Chao1, abundance-based coverage estimator (ACE), Simpson, and good coverage. These indices were calculated by the QIIME software (v1.7.0) and illustrated by the R software (v2.15.3). Redundancy analysis (RDA) was implemented through the Canoco software (v4.51; Microcomputer Power, Ithaca, NY, USA), whereas Spearman's rank correlations and variance analysis was conducted using MATLAB (v7.0, MathWorks, Inc., Natick, MA), and histogram was obtained through Microsoft Excel 2010. Raw data sequenced were deposited in the NCBI Sequence Read Archive in accordance with accession numbers SRR8196844, SRR8196845, SRR8196846, SRR8196847, SRR8196848, SRR8196849, SRR8196850, SRR8196851, SRR8196852, SRR8196853, SRR8196854, and SRR8196855.

Each OTU was designated to a functional guild using the FUNGuild version 1.0 database (Nguyen et al. 2016). The database includes three trophic modes, i.e., symbiotrophs, saprotrophs, and pathotrophs. Within these trophic modes, 12 categories are broadly referred to as guilds, including animal pathogens, plant pathogens, ectomycorrhizal fungi, foliar endophytes, arbuscular mycorrhizal fungi, ericoid mycorrhizal fungi, lichenicolous fungi, mycoparasites, lichenized fungi, undefined root endophytes, wood saprotrophs, and undefined saprotrophs. FUNGuild assigns function according to the matches at the genus and species level, together with confidence levels including "highly probable," "probable," and "possible." Functionality prediction was achieved according to the assessments given in existing research.

Results

Yield and chemical qualities of tea leaves

With the increase of continuous cropping years, the yield of tea leaves has a trend of increasing at first and then decreasing (Fig. 1a). The amino acid, caffeine, tea polyphenol, and catechin contents, which determined the aroma, color, and taste of tea leaves, were significantly declined in the 20-year-old tea orchards in comparison with the 1- and 10-year-old counterparts (Fig. 1b). In summary, the above results suggested the obvious decreases in the yield and chemical quality of tea leaves in the orchards with 20 years of continuous monoculture.

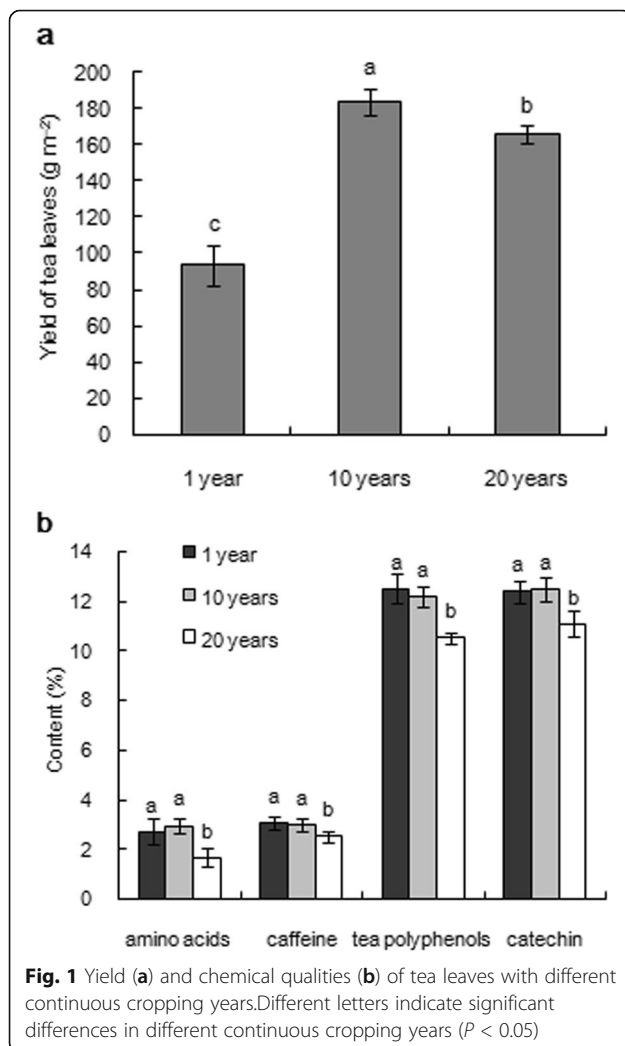
Soil chemical characteristics

Soil pH value dropped notably by 0.71, 0.99, and 1.02 from the 0-year soil to the 1-, 10-, and 20-year tea soil samples, respectively (Table 1). SOC, AN, AP, TN, and TP displayed the highest amounts in the 1-year soil,

Table 1 Chemical properties of soils from tea orchards with different continuous cropping years

Chemical properties	0 year	1 year	10 years	20 years
pH	4.88a	4.17b	3.89c	3.86c
SOC (g kg ⁻¹)	2.59c	16.58a	15.16 a	12.76b
AN (mg kg ⁻¹)	52.50c	126.70a	106.87b	107.33b
AP (mg kg ⁻¹)	5.00d	61.50a	25.00b	8.67c
AK (mg kg ⁻¹)	43.64a	26.84b	10.02c	26.84b
TN (g kg ⁻¹)	0.29b	1.46a	0.15c	0.22b
TP (g kg ⁻¹)	1.70c	6.32a	3.37b	4.23b
TK (g kg ⁻¹)	3.31b	3.31b	4.18a	4.12a
EAl ³⁺ (cmol(1/3Al ³⁺) kg ⁻¹)	2.32c	4.84b	8.03a	5.06b
EH ⁺ (cmol(H ⁺) kg ⁻¹)	0.41c	0.91b	1.47a	1.21a
EH ⁺ +EAl ³⁺ (cmol kg ⁻¹)	2.73c	5.75b	9.51a	6.27b
CEC (cmol kg ⁻¹)	4.36c	8.22ab	9.77a	7.03b

Different letters in rows indicate significant differences ($P < 0.05$).



followed by 10- and 20-years soils. The TK, EAl³⁺, EH⁺, and CEC contents in the 10-year soil samples were significantly higher than those in the other soils.

Taxonomic composition and diversity of soil fungal communities

After quality control and singleton removal, a total of 517,294 sequences were left from 12 soil samples. Among them, high-quality sequences ranged from 37,594 to 53,283 for per sample (mean = 43,108), and 90.8% of high-quality sequences were classified on the phylum level. Retrieved OTUs from 12 soil samples were principally classified in the five fungal phyla (Fig. 2a). Ascomycota was the most abundant phylum (53.35–90.82%), followed by Basidiomycota (8.27–17.60%). Zygomycota, Glomeromycota, and Chytridiomycota were the minor phyla, and their relative abundances ranged from 0.52 to 5.61%, from 0.32 to 7.70%, from 0.02 to 2.31%, respectively, across all soil samples. The relative abundances of Ascomycota followed the sequence of 0 year > 10 years > 20 years > 1 year. The relative abundances of Basidiomycota were increased continuously from the 0-year soil to the 20-year soil. The 1-year soils showed higher relative abundances of Zygomycota, Glomeromycota, and Chytridiomycota, compared with 0-, 10-, and 20-year soils.

At the order level, higher relative abundance of Pleosporales (Ascomycota) was observed in the 10- and 20-year soils compared with those in the 0- and 1-year soils (Fig. 2b). The relative abundance of Hypocreales (Ascomycota) and Trechisporales (Basidiomycota) in the 20-year soil was obviously higher than those in the 0-, 1-, and 10-year counterparts. The relative abundance of Glomerales (Glomeromycota), Mortierellales (Zygomycota),

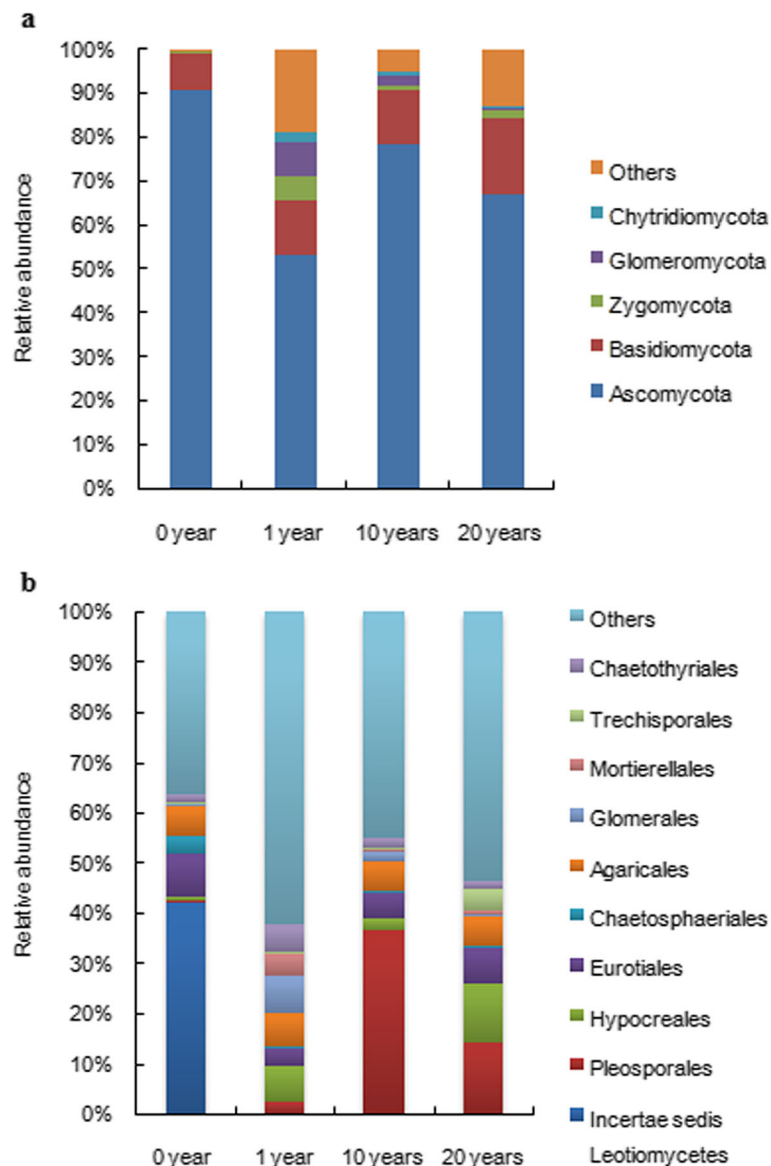


Fig. 2 Relative abundance of the dominant fungal phyla (a) and order (b) in the rhizospheric soils from tea orchards with different continuous cropping years. The “Others” comprise the unclassified and low-abundance phyla or orders

and Chaetothyriales (Ascomycota) displayed a continuous downward tendency from 1 to 20 years.

At the genus level, a total of 202 genera were found. By comparing the relative abundances of the top 30 classified fungal genera (>0.1%), only 11 fungal genera were detected with significant variations among soil samples (Table 2). The most predominant fungal genus was *Alternaria* in the 10- and 20-year soils, which were significantly higher than that in the 0- and 1-year soils. The relative abundances of *Penicillium*, *Cryptococcus*, *Cordyceps*, and *Exophiala* showed a similar upward tendency. However, the relative abundances of *Fusarium*, *Chaetomium*, *Tolyocladium*, *Claroideoglomus*, *Humicola*, and

Trichoderma showed a downward tendency continuously from 1 to 20 years.

Diversity variation was analyzed to acquire a detailed information on the structure of fungal community. The coverage of the entire samples exceeded 99%, thereby suggesting that sequencing reads were quite sufficient for the analysis (Table 3). The observed species and community richness indices (Chao1 and ACE) followed the sequence of 1 year > 10 years > 20 years > 0 year, and the Shannon and Simpson diversity indices in the 1-year soil were the highest among the four treatments, and no significant differences were found in the 0-, 10-, and 20-year soils (Table 3).

Table 2 Relative abundance of the fungal genera showing significant variation among the samples, indicated in percentage of all classified sequences

Genus*	0 year	1 year	10 years	20 years	Order	Phylum	Trophic mode
<i>Alternaria</i>	0.06c	1.89c	35.93a	13.57b	Pleosporales	Ascomycota	Pathotroph–saprotroph–symbiotroph
<i>Penicillium</i>	0.12b	2.37ab	4.40ab	5.10a	Eurotiales	Ascomycota	Saprotroph
<i>Fusarium</i>	0.10b	2.56a	0.47b	0.37b	Hypocreales	Ascomycota	Pathotroph–saprotroph
<i>Cryptococcus</i>	0.04d	1.06c	2.92a	1.96b	Tremellales	Basidiomycota	Pathotroph–saprotroph–symbiotroph
<i>Cordyceps</i>	0.00c	0.01c	0.32b	1.81a	Hypocreales	Ascomycota	Pathotroph–symbiotroph
<i>Chaetomium</i>	1.53a	1.97a	1.73a	0.15b	Sordariales	Ascomycota	Saprotroph
<i>Tolypocladium</i>	0.01c	2.23a	0.44b	0.07c	Hypocreales	Ascomycota	Pathotroph–symbiotroph
<i>Claroideoglossum</i>	0.00c	1.42a	0.59b	0.14c	Glomerales	Glomeromycota	Symbiotroph
<i>Humicola</i>	0.02b	0.67a	0.11b	0.10b	Sordariales	Ascomycota	Saprotroph
<i>Trichoderma</i>	0.16b	0.83a	0.31b	0.12b	Hypocreales	Ascomycota	Saprotroph
<i>Exophiala</i>	0.00c	0.02c	0.44b	0.80a	Chaetothyriales	Ascomycota	Saprotroph

*Only the genus with abundances higher than 0.1% are listed in the table

*Different letters within a line indicate significant differences ($P < 0.05$)

Functional groups

Overall, 14%, 29%, 54%, and 43% of OTUs from the 0-, 1-, 10-, and 20-year soils, respectively, were assigned to different functional guilds, whereas the remainders were unassigned. The three main ecological guilds (saprotrophs, symbiotrophs, and pathotrophs) were all found in the soils of four different treatments. The relative abundance of the saprotrophs in the 20-year soil (22.58%) was approximately twice of those identified in the 0-, 1-, and 10-year soils (10.62%~11.73%). The relative abundance of the symbiotrophs in the 1-year soil (8.00%) was significantly higher than those in the 0-, 10-, and 20-year soils (1.43%~2.47%). No remarkable difference in pathotrophs was found among the different treatments. Specifically, a significantly higher proportion of the pathotroph–saprotroph–symbiotroph was identified in the 10- and 20-year soils (38.85% and 15.54%) in comparison with the 0- and 1-year soils (0.11% and 2.96%) (Fig. 3).

The saprotrophs were mainly dominated by the undefined saprotroph fungi, which had a higher percentage in the 20-year tea soils in comparison with the other soil samples (Fig. 4). The symbiotrophs were mainly dominated by the arbuscular mycorrhizal fungi, which

showed a continuous downward tendency from 1 to 20 years. Finally, the pathotroph–saprotroph–symbiotroph fungi consisted principally of animal pathogen–endophyte–plant pathogen–wood saprotroph, which had a higher relative abundance in the 10- and 20-year soil samples (35.93% and 13.57%) compared with that in the 1-year counterpart (1.89%).

Effects of chemical properties on fungal community structure and function

RDA was conducted to investigate the effects of soil properties on fungal communities and function groups (Fig. 5a). A significant correlation was found between soil chemical properties and fungal communities ($P = 0.002$). The principal two axes of RDA accounted for nearly 57.8% and 27.4% of the total variance in the fungal community. The fungal communities in the 1-, 10-, and 20-year soil samples were grouped from those found in the 0-year soil along the first axis and significantly correlated with pH, SOC, and EAI^{3+} ($r = 0.9627$, -0.9053 , and -0.8859 , respectively). The 1- and 10-year communities were separated by the second axes, which was highly correlated with TN ($r = -0.6808$). pH value accounted for the maximum proportion of

Table 3 Calculations of the observed species, richness, diversity, and coverage of all samples

Treatments	Observed species	Chao1	ACE	Shannon	Simpson	Coverage (%)
0 year	317.67d	350.70d	359.36d	4.45b	0.88ab	99.83a
1 year	556.67a	710.36a	644.47a	6.40a	0.97a	99.73a
10 years	475.67b	513.46b	522.99b	4.58b	0.84b	99.80a
20 years	399.67c	440.72c	444.06c	4.97ab	0.92ab	99.80a

Chao 1, richness estimator; ACE, abundance-based coverage estimator; Shannon, nonparametric Shannon diversity index; Simpson, Simpson diversity index; Coverage, Good's nonparametric coverage estimator

Different lowercase letters (a-d) in the same column indicate statistically significant differences ($P < 0.05$)

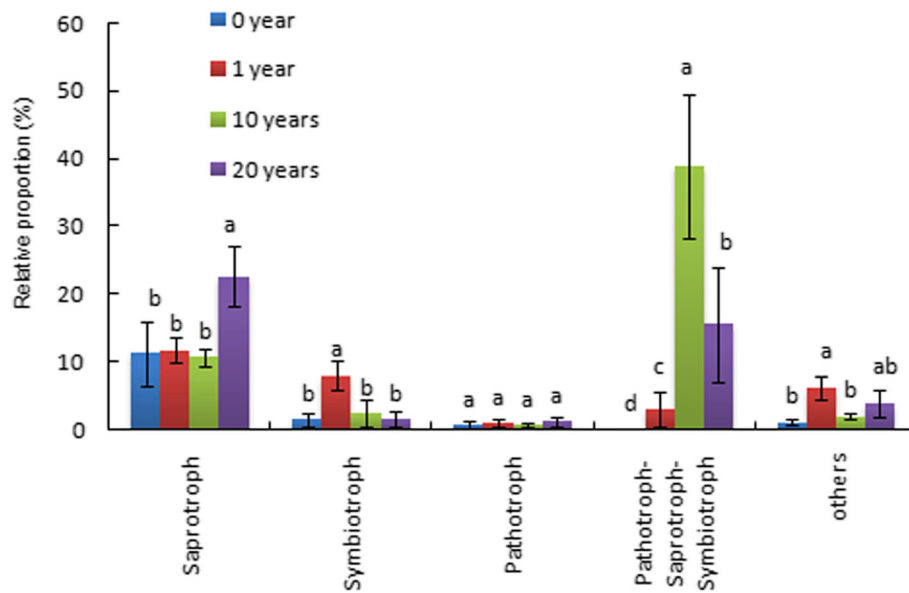


Fig. 3 Relative abundance of fungal trophic modes in the rhizospheric soils from tea orchards with different continuous cropping years

total variance in fungal communities (54.4%, $P = 0.002$), and then followed by TN (15.7%, $P = 0.026$), SOC (10.5%, $P = 0.008$), TK (6.0%, $P = 0.044$), and EAI^{3+} (4.0%, $P = 0.034$).

A significant correlation was also detected between soil properties and fungal functional groups ($P = 0.002$,

respectively). The principal two axes of RDA accounted for 78.9% and 6.3% of the total variation (Fig. 5b), and the fungal functional groups in the 0-, 1-, 10-, and 20-year soils clearly differed from one another. EAI^{3+} was detected to hold the largest proportion of total variation in fungal functional groups (56.3%, $P = 0.002$), followed

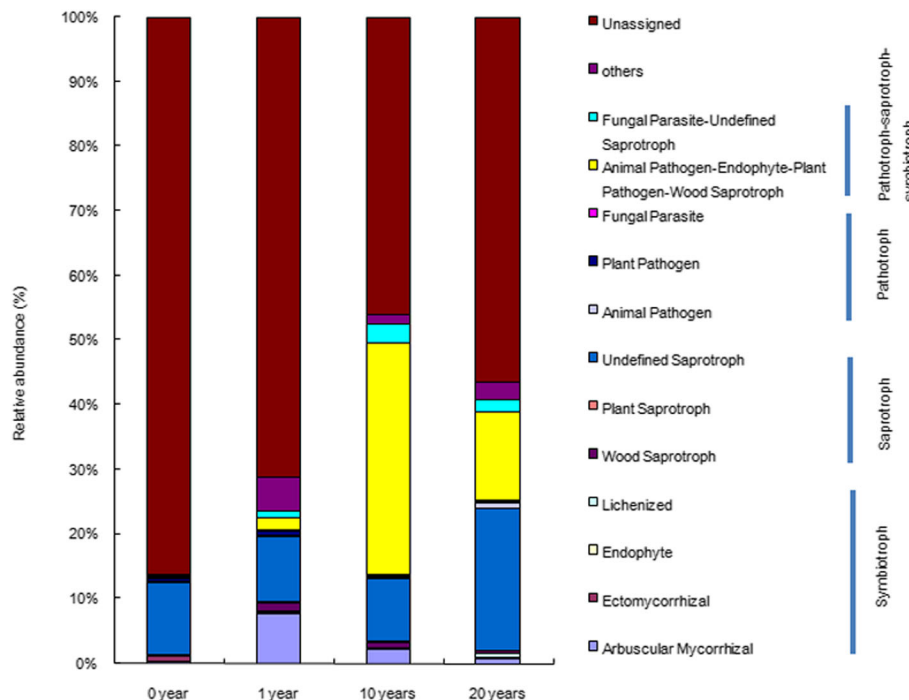


Fig. 4 Relative abundance of fungal functional groups (guilds) in the rhizospheric soils from tea orchards with different continuous cropping years

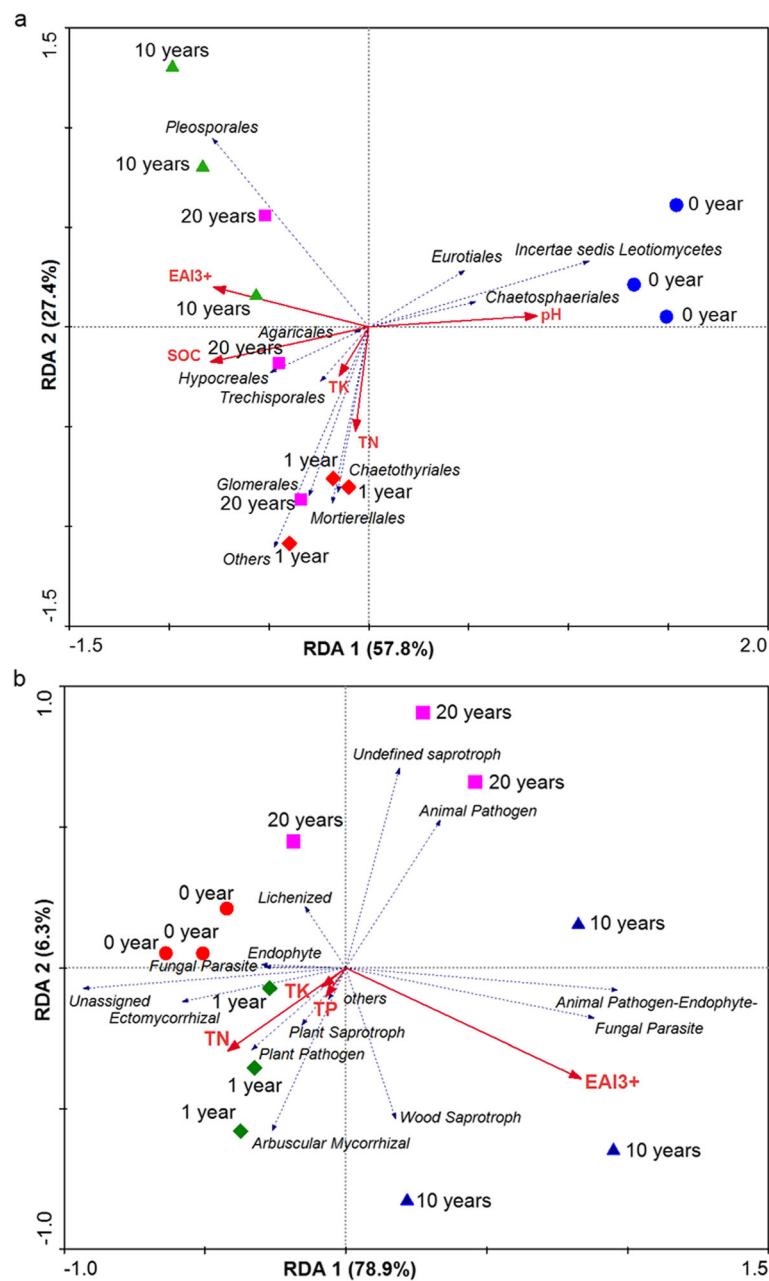


Fig. 5 RDA analysis of the fungal communities at the order level (a) and fungal functional groups (b) and soil properties

by TK (11.9%, $P = 0.038$), TN (10.5%, $P = 0.102$), and TP (9.8%, $P = 0.020$). RDA suggested that the functional groups from the 10- and 20-year soils were associated with higher EAI³⁺.

The Spearman's rank-order correlation analysis showed that the relative abundances of Ascomycota, Incertae sedis Leotiomycetes, and Chaetosphaeriales had significantly negative correlation with SOC, TP, AN, and AP, whereas the relative abundances of Zygomycota, Glomeromycota, Chytridiomycota, Glomerales, Mortierellales, and Chaetothyriales were positively correlated

with SOC, TP, AN, and AP (Table 4). The relative abundance of Pleosporales was positively related to EAI³⁺, EH⁺, CEC, and SOC, and was negatively correlated with TN. The relative abundances of *Alternaria*, *Penicillium*, *Cryptococcus*, *Cordyceps*, and *Exophiala* were positively related to EAI³⁺, EH⁺, CEC, and SOC and were negatively correlated with pH and TN. The relative abundance of *Tolypocladium*, *Claroideoglossum*, and *Humicola* were positively correlated with SOC, TP, AN, and AP. The relative abundances of symbiotroph, arbuscular mycorrhizal, and wood saprotroph fungi had

Table 4 Spearman's rank correlations coefficients between soil fungal communities and soil properties among tea orchards with different continuous cropping years

Phylum/order/genus	SOC	TN	TP	TK	AN	AP	AK	pH	EAl ³⁺	EH ⁺	CEC
Ascomycota	-0.557	-0.239	-0.860**	-0.218	-0.806**	-0.697*	0.637*	0.455	-0.406	-0.396	-0.504
Basidiomycota	0.014	-0.214	0.357	0.215	0.399	0.113	-0.428	-0.538	0.462	0.410	0.168
Zygomycota	0.427	0.561	0.769**	0.118	0.701*	0.592*	-0.417	-0.021	-0.007	-0.004	0.238
Glomeromycota	0.574	0.386	0.643*	0.147	0.620*	0.803**	-0.370	0.000	0.154	0.130	0.490
Chytridiomycota	0.928**	0.126	0.748**	-0.140	0.746**	0.951**	-0.545	-0.371	0.517	0.490	0.797**
Incertae sedis Leotiomycetes	-0.841**	-0.196	-0.762**	0.104	-0.862**	-0.965**	0.509	0.273	-0.434	-0.396	-0.720**
Pleosporales	0.620*	-0.604*	0.168	-0.183	0.315	0.444	-0.348	-0.524	0.776**	0.680*	0.699*
Hypocreales	0.469	0.098	0.755**	0.004	0.708*	0.479	-0.476	-0.455	0.301	0.291	0.273
Eurotiales	-0.329	-0.354	-0.455	-0.021	-0.606*	-0.528	0.234	-0.084	-0.119	0.000	-0.301
Chaetosphaeriales	-0.699*	-0.261	-0.782**	0.246	-0.777**	-0.781**	0.613*	0.305	-0.302	-0.344	-0.495
Agaricales	-0.049	0.175	0.042	-0.140	0.081	0.014	-0.384	0.140	-0.007	-0.144	0.000
Glomerales	0.637*	0.337	0.594*	0.047	0.609*	0.838**	-0.355	0.035	0.182	0.137	0.538
Mortierellales	0.427	0.561	0.769**	0.118	0.701*	0.592*	-0.417	-0.021	-0.007	-0.004	0.238
Trechisporales	0.202	-0.356	0.058	0.080	0.211	0.059	0.135	-0.469	0.444	0.481	0.306
Chaetothyriales	0.410	0.607*	0.664*	-0.011	0.623*	0.627*	0.048	0.196	-0.210	-0.273	0.049
<i>Alternaria</i>	0.582*	-0.619*	0.144	-0.204	0.268	0.395	-0.354	-0.515	0.778**	0.663*	0.651*
<i>Penicillium</i>	0.560	-0.540	0.315	-0.143	0.305	0.359	-0.458	-0.685*	0.692*	0.729**	0.573
<i>Fusarium</i>	0.473	-0.137	0.420	-0.111	0.592*	0.500	-0.501	-0.294	0.552	0.308	0.469
<i>Cryptococcus</i>	0.519	-0.742**	0.207	0.063	0.230	0.349	-0.554	-0.809**	0.932**	0.923**	0.746**
<i>Cordyceps</i>	0.205	-0.616*	0.180	0.058	0.180	0.028	-0.233	-0.780**	0.638*	0.733**	0.310
<i>Chaetomium</i>	0.312	0.320	0.109	-0.161	0.119	0.356	-0.334	0.466	-0.081	-0.196	0.228
<i>Tolypocladium</i>	0.827**	0.189	0.797**	-0.054	0.813**	0.937**	-0.589*	-0.238	0.420	0.329	0.671*
<i>Claroideoglomus</i>	0.765**	0.184	0.803**	0.076	0.832**	0.950**	-0.553	-0.275	0.430	0.360	0.676*
<i>Humicola</i>	0.508	-0.091	0.587*	0.168	0.704*	0.613*	-0.505	-0.413	0.566	0.382	0.545
<i>Trichoderma</i>	0.644*	0.344	0.538	-0.129	0.564	0.754*	-0.311	0.126	0.091	0.028	0.462
<i>Exophiala</i>	0.281	-0.570	0.365	0.162	0.309	0.180	-0.298	-0.870**	0.684*	0.736**	0.368

* $P < 0.05$; ** $P < 0.01$ (Spearman's rank correlation coefficient test)

significantly positive relationship with SOC, TP, TP, and AN (Table S1). The relative abundance of pathotroph–saprotroph–symbiotroph was positively related to EAl³⁺, EH⁺, CEC, and SOC, and was negatively correlated with pH and TN

Discussion

Soil chemical characteristics in the tea orchards

Long-term monoculture of tea plants generally causes a decrease in soil pH and an increase in the Al toxicity (Wang et al. 2010; Arafat et al. 2017). In the present study, continuous cropping of tea plants resulted in the expected low soil pH and high Al³⁺ concentration. Except for AK and TK, the soil nutrition contents became depleted after long-term continuous cropping, which was in agreement with the previous experimental results (Dang 2002). In addition, the yield and quality of tea leaves became lower in the 20-year tea orchard. Therefore, the growth of tea plants was suppressed, and soil

quality became worse after the long-term continuous monoculture of tea orchard.

Fungal community structure, function, and diversity

Illumina HiSeq sequencing analysis of ITS2 sequences revealed that long-term continuous monoculture of tea plants changed the structure of the soil fungal community. The most abundant fungal phyla were Ascomycota and Basidiomycota in all soil samples, which is in agreement with a previous research (Schmidt et al. 2013). It has been reported that Ascomycota have capability to tolerate stressful conditions such as low nutrient availability to achieve a more efficient resource use in challenging environment (Chen et al. 2017). In this study, the proportion of Ascomycota was lowest in the 1-year soils (Fig. 2a); this was probably the results of the rich nutrition substrates found in the 1-year soils (Table 1). Conversely, 0-, 10-, and 20-year soils showed higher relative abundance of Ascomycota and this may due to the lower

soil nutrition substrates. This hypothesis was supported by the Spearman's rank correlation analysis which indicated that Ascomycota had significantly negative correlation with TP, AN, AP, and AK (Table 4). The relative abundance of Basidiomycota increased with extension in continuous cropping years, which showed consistent variation with the soil fungal community of differently aged forests (Han et al. 2016). Basidiomycota plays a significant role in the degradation of high-lignin content plant litter (Guo et al. 2018). Tea plants require periodic pruning to maintain tea bush height and increase production by promoting branching. In the process of litter decomposition, readily degradable substances (e.g., cellulose) were rapidly decomposed, and the recalcitrant substances such as lignin were left behind (Marschner et al. 2011). With the increase of stand age, more lignin were accumulated in the tea soils, probably stimulating the positive growth and reproduction of Basidiomycota (Baldrian et al. 2011, Yang et al. 2019).

Furthermore, we found that long-term continuous ratoon cropping of tea plants led to a significant increase in the relative abundance of *Alternaria*, *Penicillium*, and *Exophiala* in Ascomycota phylum. The genus *Alternaria* are known as major plant pathogens causing 20–80% agricultural losses in field crops, horticultural crops, plantation crops, forest plants, and also postharvest storage (Nagrale et al. 2016). In this study, *Alternaria* (pathotroph–saprotroph–symbiotroph fungi) was the most abundant genus, accounting for 35.93% and 13.57% of the total fungal genera in the 10- and 20-year soils, respectively. The increased proportion of *Alternaria* in the 10- and 20-year soils could increase the risk of plant disease outbreak and cause considerable yield loss (Leiminger et al. 2015). The genus *Penicillium* are well-known to solubilize phosphates (Wakelin et al. 2004) and to excrete antifungal substances that potentially hinder the growth of plant pathogenic fungi (Yang et al. 2008). In this study, the increase of genus *Penicillium* from the 0- to 20-year soils may be related to the dynamics of available phosphate and pathogenic microorganisms with increasing continuous cropping years. The genus *Exophiala* are commonly identified as saprobes existing in the soil and water environments, opportunistic pathogens inhabiting the animals, or endophytes surviving in the plant roots (Cheikh-Ali et al. 2015). By contrast, *Trichoderma* and *Chaetomium* in Ascomycota phylum showed a significant decrease in the 10- and 20-year soils, which have been well-known to suppress plant pathogen infection and are putatively plant-beneficial fungal groups (Park et al. 2005; Bailey et al. 2006).

Generally, symbiotrophic fungi are extremely beneficial to the health, nutrition, and quality of most crops (Rouphael et al. 2015; Igiehon and Babalola 2017). Pathotrophic fungi generally derive nutrient substance

by attacking host cells; thus, they are considered to cause disease or perform the negative effects on plant performance (Anthony et al. 2017). In this study, 1-year tea soils had a higher proportion of symbiotrophs compared with the 10- and 20-year soils, whereas a higher abundance of pathotroph–saprotroph–symbiotroph fungi was identified in the 10- and 20-year tea soils in comparison with the 0- and 1-year tea soils, indicating that tea plants might suffer a higher risk of soil-borne fungal diseases with extension in continuous cropping years.

Soil microbial richness and diversity play an essential role in maintaining the soil quality and the function and sustainability of a soil ecosystem (Garbeva et al. 2004). Thus, the loss of microbial richness and of diversity in tea soils is probably one of the reasons that resulted in the poor growth of tea plants. Fungal diversity was decreased significantly in the 10- and 20-year tea soils compared with that in the 1-year soil, indicating that tea continuous cropping may have decreased the fungal diversity through the 10 or 20 years of plantation.

Relationship between microbial characteristics and soil properties

Soil properties, such as SOC and pH value, are key influencing factors of fungal community structure (Liu et al. 2015; Ding et al. 2017). Soil pH may affect fungal community structure by changing nutrient availability or imposing some physiological constraints on fungal growths (Marschner et al. 2005). In the present study, pH value was found as the most important factor for the fungal communities, followed by TN and SOC (Fig. 5a). This finding was consistent with previous observations that the fungal community significantly changed following soil pH (Siles and Margesin 2016). Moreover, Spearman's rank correlation analysis also indicated that some fungal genera such as *Penicillium*, *Cryptococcus*, *Cordyceps*, and *Exophiala* were significantly affected by soil pH (Table 4). In addition, most fungi belong with heterotrophs and are dependent on exogenous carbon supply for their growth; consequently, SOC may affect fungal community structure (Broeckling et al. 2008). For fungal functional groups, EAI^{3+} was the most important factor (Fig. 5b). He et al. (2014) reported that high aluminum concentration reduced the richness and diversity of soil fungi and affected the fungal community composition. The Spearman's rank correlation analysis also indicated that pathotroph–saprotroph–symbiotroph fungi were significantly affected by EAI^{3+} (Table S1). The fungal community compositions were also sharply influenced by the root system (Philippot et al., 2013; Mendes et al., 2014). Tea roots grown for certain years probably secrete exudates that include antimicrobial metabolites (Pandey and Palni 1996), and the influence of the root exudates on fungal community distribution in

rhizospheric soils of tea plants need to be further studied.

In conclusion, long-term continuous monoculture of tea plants shifted the composition and functional groups in soil fungal communities. There was a significant increase in the abundance of potential pathogen *Alternaria* (pathotroph–saprotroph–symbiotroph fungi) in the soils of 10- and 20-year tea orchards. In contrast, there was a significant decrease in the relative abundance of beneficial symbiotrophic fungi with extension in continuous cropping years. The richness and diversity indices of the fungal community also significantly declined with long-term continuous cropping. Analysis of the effects of soil properties on fungal community structure and function clearly revealed that pH value and EAI³⁺ showed their strongest effects on fungal community structure and functional groups, respectively. Results can provide basis for future studies on sustainable agricultural measures to address the long-term continuous monoculture problems of tea plants.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13213-020-01555-y>.

Additional file 1: Table S1. Spearman's correlation analysis of fungal trophic modes and guilds, and soil properties among tea orchards with different continuous cropping years

Authors' contributions

Yanchun Li and Zhaowei Li carried out all experiments and wrote this manuscript. Yasir Arafat contributed to the statistical analysis. Wenxiong Lin designed the experiments and revised this manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This article does not contain any studies performed by any of the authors with human participants.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interest

The authors declare that they have no competing interests.

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