Influence of tea-Pleurotus ostreatus intercropping on soil fungal diversity and community structure1

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ARTICLE

Influence of tea-*Pleurotus ostreatus* intercropping on soil fungal diversity and community structure[1]

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Abstract: Understanding the relationship between soil fungal communities and soil function is vital to establish a sustainable and ecologically friendly tea (*Camellia sinensis* L.) cultivation. However, there is limited research on the response of soil fungal communities to tea-fungus intercropping, particularly how it is related to soil biodiversity and fertility. Here, we assessed and compared the fungal communities using a metabarcoding technique, soil properties in three plantations (1, 2, and 5 yr of tea-*Pleurotus* intercropping plantations), and a 5 yr chemically fertilized monoculture plantation. We obtained a total of 3493 operational taxonomic units (OTUs) from four tea plantations. Five hundred and ninety-three fungal OTUs are shared by all plantations, and the other 471 fungal OTUs are shared by three plantations. The largest number of OTUs was recorded in 5 yr tea-*Pleurotus* intercropped plantations ($N = 2040$), followed by 2 yr ($N = 2024$) and then 1 yr ($N = 1471$), while the chemically fertilized plantation recorded 1823 OTUs. Tea-*Pleurotus* intercropping showed a significant effect on the increased diversity of soil fungal diversity compared with the monoculture tea cultivations. Fungal groups Basidiomycota, Ascomycota, and Mortierellomycota were the most abundant taxonomic groups recorded in all soil samples. Principal coordinate analysis revealed that fungal community composition in tea-*Pleurotus* intercropped plantations and monoculture tea plantations was significantly different. Besides, redundancy analysis revealed that soil nutrients significantly influence soil fungal community composition. Our results demonstrate that tea-*Pleurotus* intercropping may offer long-term benefits to soil biodiversity and sustainable benefits in the tea plantations.

Key words: co-friendly plantations, tea-fungus intercropping, fungal communities, metabarcoding.

Résumé : Il est crucial de comprendre ce qui relie les populations telluriques de cryptogames au fonctionnement du sol si l'on veut parvenir à une culture durable et écologiquement saine du thé (*Camellia sinensis* L.). Pourtant, on effectue peu de recherches sur la réaction des populations fongiques du sol à la culture intercalaire du thé et de champignons, plus précisément ses rapports avec la biodiversité et la fertilité du sol. Les auteurs ont évalué et comparé les communautés fongiques en recourant à la technique du méta code à barres ainsi que les propriétés du sol dans trois plantations (cultures intercalaires de thé et de pleurote d'un, de deux et de cinq ans) et une monoculture du thé de cinq ans, fertilisée avec des engrais chimiques. Les quatre plantations ont globalement donné 3493 unités taxonomiques opérationnelles (UTO). Elles partageaient toutes 593 UTO fongiques, les 471 restantes se retrouvant dans trois d'entre elles. Le plus grand nombre d'UTO a été relevé dans la plantation thé-pleurote de cinq ans ($N = 2040$). Suivaient la plantation de deux ans ($N = 2024$) et celle d'un an ($N = 1471$). La plantation fertilisée avec des engrais chimiques comptait 1823 UTO. La culture intercalaire de thé et de pleurote accroît sensiblement la diversité des cryptogames dans le sol, comparativement à la monoculture. Les basidiomycètes, les ascomycètes et les mortierellomycètes sont les groupes les plus abondants découverts dans

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les échantillons de sol. L'analyse en coordonnées principales (PCoA) révèle de nettes différences dans la population de cryptogames entre les cultures intercalaires thé-pleurote et la monoculture du thé. D'autre part, l'analyse par redondance indique que les oligoéléments dans le sol exercent une influence significative sur la composition de la population de cryptogames. Ces résultats donnent à penser que la culture intercalaire du thé et du pleurote pourrait avoir des bienfaits à long terme sur la biodiversité du sol et présenter des avantages pour la culture durable du thé. [Traduit par la Rédaction]

Mots-clés : plantations écologiques, culture intercalaire de thé et de champignons, populations fongiques, méta code à barres.

Introduction

Tea (*Camellia sinensis* L.) is an important global industry, which is widely grown from the tropical to the subtropical areas of some Asian countries, such as southern China (Kavitha et al. 2016). By 2020, the total land area for tea plantations in China was estimated at around 2.93 million ha, accounting for more than half of the world's total tea planting area and producing 2.61 million tons of tea (about 45% of the world's total tea production) in 2018 alone. However, drastic decline in yield, soil degradation, and reduction of beneficial soil bacteria were observed in long-term monoculture tea cultivations, which concerns many soil ecologists and agriculturists (Han et al. 2007; Li et al. 2016; Arafat et al. 2017; Gu et al. 2019). The monoculture mode in tea plantations also poses more environmental concerns toward soil erosions (Zhang et al. 2003).

Plantation intercropping is commonly used in China for multiple crop cultivation because of its positive benefits on reducing the risk of crop failure by improving land-use management (Seran and Brintha 2010; Song et al. 2021). Compared with monoculture plantations, intercropping mode of cultivation can improve soil quality by adding a large amount of topsoil and subsoil organic matter, which bolsters the releasing and recycling of soil nutrients (Zeng 2009; Powlson et al. 2011; Rowe et al. 2016). Intercropping cultivation provides retentive ground cover that promotes soil structure and prevents drastic erosion in monoculture plantations (Zeng 2009; Gebru 2015). Therefore, intercropping is considered a suitable agricultural practice for the long-term maintenance of soil fertility and optimization of this system compared with traditional monoculture cultivation (Gebru 2015; Song et al. 2021). Among intercropping strategies fungus-plant intercropping has been recently practised in different Chinese plantation systems and showed beneficial effects on soil fungal communities and properties (Chen et al. 2012; Song et al. 2021). Song et al. (2021), for example, reported the positive effects of peach-*Morchella* intercropping in soil properties and fungal communities, whereas pear-*Pleurotus* intercropping showed increase in soil microbial community, soil fertility, and fruit yield quality (Chen et al. 2012). In tea plantations, tea-*Stropharia rugosoannulata* intercropping was previously studied and significantly increased soil organic matter content, total and available N content, and reduced soil acidification and soil bulk density, and consequently improved soil structure (Han and Chen 2013). Similarly, intercropping a different fungal species, *Oudemansiella radicata*, in tea plantations has shown a significant increase in the soil available N, P, and K content and tea polysaccharide content (Jing et al. 2018). Other studies have also found that fungal intercropping in tea plantations enriched the abundance of bacteria and actinomycete in soil compared with monoculture tea plantations (Yang et al. 2017).

Fungi are ubiquitous and vital part of overall soil ecosystems; therefore, improving fungal diversity in soils plays a key role in optimizing critical ecological function including nutrient cycling, organic matter decomposition, and plant primary productivity (Klaubauf et al. 2010; Tedersoo et al. 2014; Cobb et al. 2016; Saha et al. 2019). Previous studies showed that the composition and diversity of soil fungal communities are influenced by various factors such as plant species composition, soil types, and agricultural management (Guo et al. 2016; Trivedi et al. 2016; Zhang et al. 2016; Carson et al. 2019). By understanding the role of soil fungal communities in soil ecosystems especially in agroecosystems, it is critical to highlight their ecosystem services and benefits (Delgado-Baquerizo et al. 2016). Previous studies on this system mainly focused on the effects of intercropping edible fungus on soil properties soil and tea yield. Yet, there is a relative lack of studies on the composition and diversity of the soil fungal community in the tea-fungal intercropping plantations, which warrants in-depth studies and investigations. Second to *Agaricus bisporus*, the edible mushroom *Pleurotus ostreatus* (Jacq. Fr.) Kummer is a widely grown fungal taxa mainly for food and other industrial use making it an ideal fungal intercrop in tea plantations (Sánchez 2010). *Pleurotus ostreatus* persists in a wide range of environments because of its low environmental requirements enabling its easy colonization of wide suite of lignocellulosic substrates and other waste products, especially from agroecosystems. Additionally, *P. ostreatus* has a shorter growth period and simple sterilization (Das and Mukherjee 2007) making it inexpensive and viable fungal intercrops in tea cultivations.

Precise assessment of fungal communities in soil ecosystems is important to fully understand impacts of different systems, yet this is time consuming and challenging (Begerow et al. 2010). High-throughput metabarcoding techniques have been recognized as a powerful tool to study fungal communities in ecosystems due to their high resolution and efficiency in detection (Li et al. 2016; Yang et al. 2019; Joos et al. 2020; Yang et al. 2021). In this study, high-throughput sequencing technology was used to assess the composition and diversity of soil fungal communities in tea-*Pleurotus ostreatus* (hereinafter tea-*Pleurotus*) intercropping with 1, 2, and 5 yr cultivation time versus monoculture tea plantations with 5 yr chemical fertilizer treatment in the hilly region of southwest Yunnan, China. The study further aimed to explore the relationship between changes in fungal communities and soil parameters to explore the potential environmental factors that influence the variation in diversity. Consequently, we hope that our findings will provide a basis for the establishment of organic and ecologically friendly tea cultivation using tea-*Pleurotus* intercropping in southwest China.

Materials and Methods

Tea-*Pleurotus* intercropping

We sampled soils from tea plantations of Pu'er City in the province of Yunnan, southwest China. The location lies in the low-latitude plateau of the southern subtropical monsoon climate zone, with an altitude of about 1302 m a.s.l. The annual average temperature ranges from 15 °C to 20 °C and the annual precipitation ranges from 1200 to 2700 mm. The cultivated tea variety in Pu'er is the big leaf tea, which was planted in 2004. The edible fungus used for intercropping is *P. ostreatus* (cultivar PG981; each bag has 500 g of fungus inoculant).

We collected soil samples for the experiment from four treatment plot areas of the plantations, namely: the 1 yr intercropping plantation (I1), the 2 yr tea-fungus intercropping plantation (I2), and the 5 yr tea-fungus intercropping plantation (I3). Monoculture plantation with chemical compound fertilizer applied for 5 yr (M1) with CK. The management measures and the environmental condition of all the plantations sampled are consistent and similar throughout the experiment. Edible fungi were inoculated in the tea cultivations. First, the surface of tea rows was cleared from weeds before *P. ostreatus* was inoculated in the soil. The fungi were intercropped in the wider side of the tea row, by digging a trench with a width of about 20 cm and depth of about 18 cm at a distance of 10–15 cm from the stem of the tea plant to avoid damaging the main root system of the plant. The bag of *P. ostreatus* inoculant was stripped and positioned horizontally in the trench to allow the fungus bag and the soil rhizosphere tightly placed on the side of the tea row. Fungal bags are connected from end to end of a row and then covered with 2–3 cm soil. The setup was consistently irrigated to ensure that the fungal bags is moisturized at about 60%. Under suitable conditions, the edible fungus can generally be harvested for three to five cropping seasons. Edible fungi are typically planted from August to October every year.

Soil sampling and collection

Each soil sample was represented by four samples collected around each sampling point. We collected two contiguous soil samples (i.e., each for soil fungal community analysis and soil chemical analysis) using steel cores of 5 cm diameter and 9–10 cm depth for fungal diversity and soil nutrient analysis. The sampling depth of the cores was in the deeper 10–30 cm layer to maximize the detection of fungal communities (e.g., Zhang et al. 2020). Soils for microbial samples were thoroughly sieved using a 2 mm mesh, homogenized, and then divided into two subsamples: a 5 g of homogenized soil was used for molecular analysis to evaluate the soil microbial community. Fifty grams of air-dried soil from the same samples was used for soil physicochemical analysis that includes total carbon (TC), total N (TN), total P (TP), total K (TK), hydrolyzed N (HyN), available P (AP), available K (AK), and organic matter (OM). These soil composition parameters were measured using the standard analysis procedure at the central laboratory of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (XTBG-CAS), Menglun, P.R. China.

Microbial diversity analysis

DNA extraction and PCR amplification

We used the Biofast Soil Genomic DNA Extraction Kit (TIANamp Soil DNA Kit, Germany) to extract three genomic DNA from 5 g subsamples according to the manufacturer's instructions. To minimize DNA extraction bias, three DNA extractions from each sample were combined prior to PCR. The combined DNA was purified using DNA Purification Kit (TIANgel Purification Kit, Germany) and then stored at −20 °C. Fungal DNA was initially amplified with the primers: ITS1F (5′-`CTTGGTCATTTAGAGGAAGTAA`-3′) and ITS2 (5′-`GCTGCGTTCTTCATCGATGC`-3′) for library preparation. The PCR reactions were repeated three times to minimize PCR bias (e.g., in Liu et al. 2013; Yu et al. 2012) in a 20 μL mixture containing 4 μL 5 × FastPfu Buffer, 2 μL 2.5 $\text{mmol}\cdot\text{L}^{-1}$ dNTPs, 0.5 μL each primer (5 $\mu\text{mol}\cdot\text{L}^{-1}$), 0.4 μL FastPfu Polymerase, and 10 ng template DNA, and makeup 20 μL with purified water. PCR reaction conditions are 95 °C pre-denaturation for 4 min; 94 °C denaturation for 30 s, 45 °C annealing for 20 s, 65 °C extension for 30 s, 5 cycles; denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, extension at 72 °C, 30 s, 20 cycles; extension at 72 °C for 4 min; storage at 10 °C.

Library establishment and Illumina Miseq sequencing

After quantitative mixing of PCR products, we utilized the library building kit provided by Illumina (Illumina Inc., California, United States) to build the reference library according to the building instructions and performed absolute quantification of the library using the

2 × SYBR provided by Beijing Soleibao Technology Co., Ltd. After mixing the purified library in approximately equimolar concentrations, we used MiSeq ReagentKit v2.5 (Illumina Inc. 2015) for paired-end sequencing (2 × 250).

Illumina data analysis

The quality of the sequences was first detected with FastQC software, and quality score is greater than 20, then used the default pipeline in QIIME to control the quality through the following Caporaso et al. (2010): (*i*) the reads were truncated in two ends to achieve average quality value less than 20 bp and discard the trimmed sequence that was less than 50 bp; (*ii*) the reads with two nucleotide mismatches in the primer sequence and ambiguous bases were removed; (*iii*) when the sequence is assembled, only sequences with an overlap of more than 10 bp are assembled according to the overlapping sequence, and the reads that cannot be assembled with less than 10 bp were discarded; (*iv*) sequences shorter than 300 bp after assembly were eliminated. Fungal sequences were clustered into operational taxonomic units (OTUs) with a 97% identity threshold, and the UPARSE pipeline was used to select OTUs (Edgar 2013). Using UCHIME, chimeric sequences were identified and removed. Rarefaction curves, the abundance-based coverage estimators (ACE), Chao1, Shannon–Wiener index, and Simpson's index were calculated and built based on OTUs. Chao1 and ACE were calculated to estimate the abundance of fungal communities based on sequence differences. Shannon–Wiener index and Simpson index were used to estimate the diversity within each sample (Lim et al. 2010).

Statistical analysis

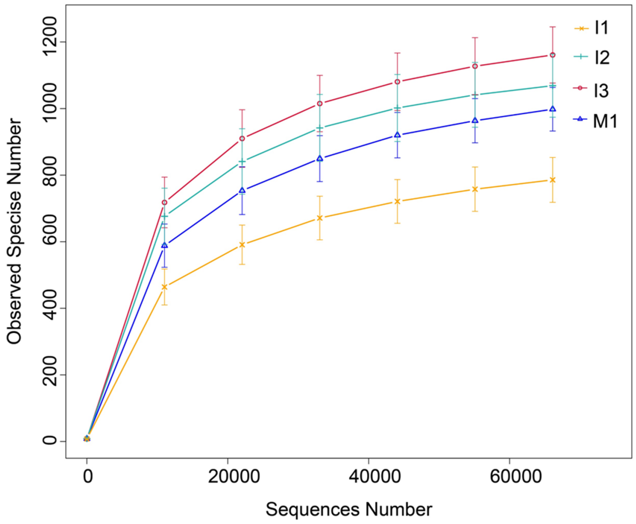
Statistical analyses and visualization were performed in R environment (version 4.02). To evaluate differences in fungal diversity and soil characteristics at different tea cultivation, we used multiple comparisons of Tukey's HSD in the "vegan" package. Principal coordinate analysis (PCoA) based on Bray–Curtis similarity distance was applied to compare fungal community similarities at different tea plantations. In addition, the similarities and differences among the communities were visualized using the number of shared and unique OTUs in the four different plantations using the Venn diagram. We compared the top 10 classified phyla and 30 classified genera using heat map analysis in the "pheatmap" package. Lastly, the soil fungal composition and properties were categorized by redundancy analysis (RDA) and using the Monte Carlo permutation test to determine the significance of the RDA axis using the "ade4" package.

Results

Soil fungal community and diversity

The sequencing coverage of soil samples in the four plots reaches from 99.75% up to 99.83% as the depth of sequencing increases, which sufficiently covers the measurable sequences in the samples (Fig. 1). A total of 3493 OTUs were identified from all tea plantations sampled. There were 1471, 2024, 2040, and 1823 OTUs in the soil samples from treatments I1, I2, I3, and M1(CK), respectively. There were 593 fungal OTUs shared by all plantations and 471 fungal OTUs shared by three plantations (Fig. 2). The 5 yr tea-fungal intercropping plantation showed the highest abundance (ACE and Chao1), while soils from the 2 yr tea-*Pleurotus* intercropping plantations had the highest richness (Shannon and Simpson index). Yet, the values of the Simpson index were not significantly ($P > 0.05$) differ across the four plantations (Fig. 3), whereas abundance-based indices, Chao1 and ACE, showed a significant positive trend with tea-*Pleurotus* intercropping years ($P < 0.05$), but the values of diversity index Shannon and Simpson within tea-*Pleurotus* intercropping years showed inconsistent patterns (Fig. 3).

Fig. 1. Rarefaction curve for the total fungal community at different plantation types: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). [Colour online.]



Composition of soil fungal communities

The taxonomic sequences from the soils of tea plantations predominantly consist of Ascomycota (44.7%), Mortierellomycota (17.7%), and Basidiomycota (11.4%), which accounts for 73.8% of the total composition of fungal communities, while the remaining sequences either belong to smaller fungal phyla or unclassified taxa (Fig. 4A). The most representative genus *Saitozyma* was the most dominant taxa among tea plantation sites, and particularly higher in the 5 yr chemically fertilized plantations. In addition, we also identified the genera *Archaeorhizomyces, Mortierella, and Fusarium* dominating the

Fig. 2. A Venn diagram showing the number of shared operational taxonomic units across different soil samples from tea-*Pleurotus* intercropping: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). [Colour online.]

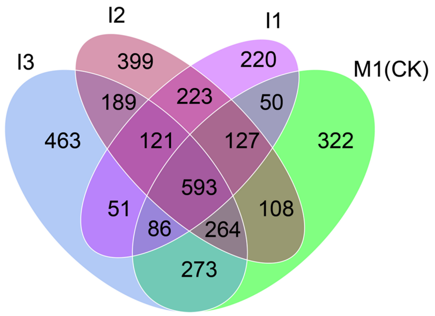


Fig. 3. Comparison of indices of richness and diversity of fungal taxa in different tea plantations: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). According to the Tukey's HSD test, values with different lowercase letters in the column (mean ± SD, $n = 4$) are significantly different at $P < 0.05$.

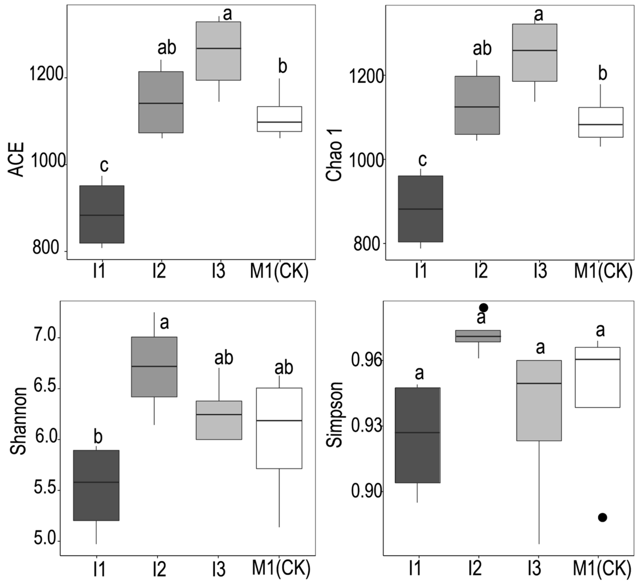
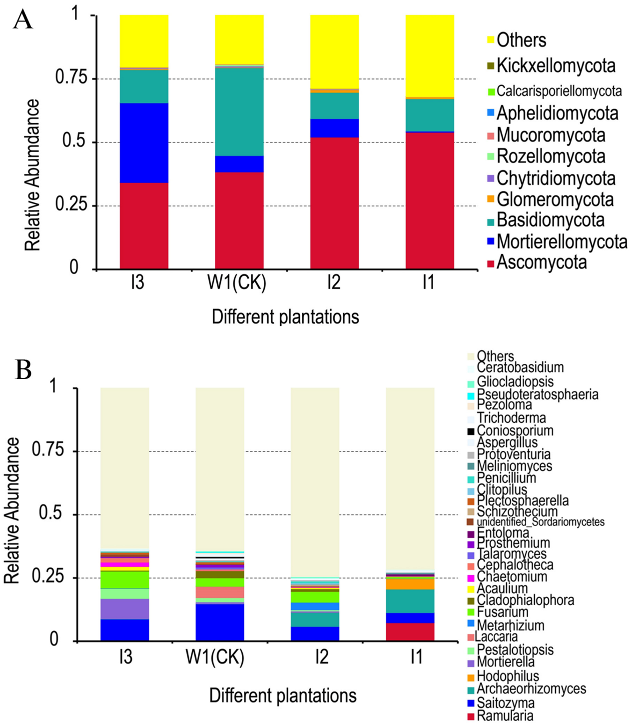


Fig. 4. The relative abundances of fungal phyla (left) and genera (right) in four tea plantations: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). [Colour online.]



tea four plantations (Table 1, Fig. 4B). Among the identified taxa at the genus level, *Saitozyma, Archaeorhizomyces, Mortierella, Fusarium,* and *Cladophialophora* showed significant differences across tea cultivation treatments. Principal coordinate analysis was employed to determine differences in the fungal community in the soil sampled from four tea cultivation treatments (Fig. 5). The first two principal coordinates represented 22.74% (PCo1) and 12.77% (PCo2) of the total variation in the fungal community in a single soil sample (PERMANOVA, $R^2 = 0.569$, $P = 0.001$). The samples in the tea-*Pleurotus* cluster showed high differences in community structure, while the PCo2 showed that the fungal communities in the intercropping tea plantations were separated with the chemically fertilized tea cultivations (Fig. 6).

Effects of soil properties on soil fungal communities

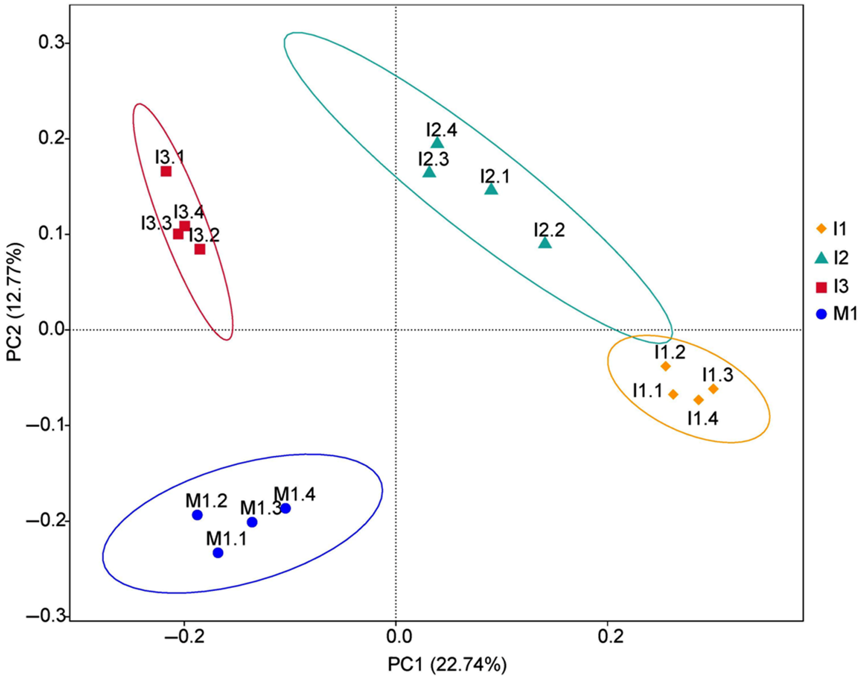
The total C, N, P, K, hydrolyzed N, available K, and organic matter content of soil showed a positive trend with the increase of the intercropping cultivation years, but the available P content showed inconsistent trend across years of intercropping period (Table 2). Among all tea-*Pleurotus* cultivation treatments, the 5 yr intercropping showed significantly higher values for the total C, N, P, hydrolyzed N, available K, and organic matter content of the soil compared with the other three plantations (Table 2). The RDA results showed that total C, N, P, K, hydrolyzed N, available K, and organic matter have a significant influence on the soil fungal communities, while the effects of available P are slightly relative (Table 3). We found a positive correlation between the diversity of Aphelidiomycota, Mortierellomycota, Entomophthoromycota, and Zoopagomycota with the total C, N, P, hydrolyzed N, available K, and organic matter content (Table 3, Fig. 6), whereas Mucoromycota,

Table 1. Relative abundance (%) of soil fungal taxa (at genus level) from different tea plantations.

Fungal taxa	I1	I2	I3	M1(CK)	Mean
Other fungal taxa	0.744 ± 0.119a	0.792 ± 0.083a	0.719 ± 0.074a	0.721 ± 0.106a	0.744
Saitozyma	0.040 ± 0.013c	0.058 ± 0.009bc	0.088 ± 0.021b	0.148 ± 0.054a	0.083
Archaeorhizomyces	0.093 ± 0.067a	0.056 ± 0.051ab	0.001 ± 0b	0.001 ± 0b	0.038
Fusarium	0.007 ± 0.001c	0.042 ± 0.011b	0.067 ± 0.018a	0.033 ± 0.022b	0.037
Mortierella	0.001 ± 0b	0.005 ± 0.003b	0.080 ± 0.054a	0.007 ± 0.005b	0.023
Ramularia	0.074 ± 0a	0.000 ± 0a	0.000 ± 0a	0.000 ± 0a	0.019
Pestalotiopsis	0.000 ± 0a	0.005 ± 0a	0.039 ± 0a	0.016 ± 0a	0.015
Laccaria	0.000 ± 0b	0.000 ± 0b	0.000 ± 0b	0.045 ± 0.005a	0.011
Cladophialophora	0.001 ± 0b	0.011 ± 0.010b	0.004 ± 0b	0.028 ± 0.014a	0.011
Hodophilus	0.041 ± 0.007a	0.000 ± 0a	0.000 ± 0a	0.000 ± 0a	0.010
Metarhizium	0.000 ± 0a	0.030 ± 0.005a	0.002 ± 0.001a	0.000 ± 0a	0.008

Note: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). Values with different lowercase letters in the column (mean ± SD, $n = 4$) are significantly different at $P < 0.05$.

Fig. 5. Principal coordinate analysis plot showing the variation across all plantations: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). The first two axes are shown as the percentage of the total variance. [Colour online.]



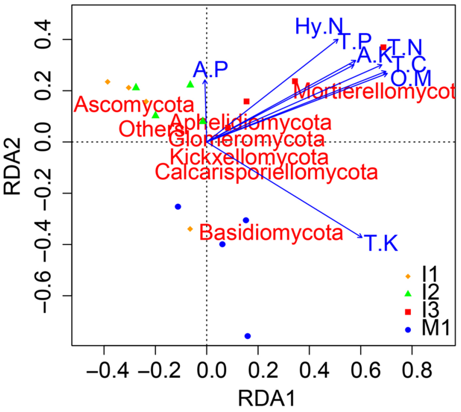
Basidiomycota, Chytridiomycota, Rozellomycota, and Blastocladiomycota were positively correlated with both total and effective K content. Calcarisporiellomycota, Monoblepharomycota, Ascomycota, and Glomeromycota were positively correlated with available P content and negatively correlated with the total K content. Overall, the highest correlation between soil fungal composition and soil properties was observed in the 5 yr tea-*Pleurotus* intercropping plantation (Table 3, Fig. 6).

Discussion

Effect of tea-fungus intercropping on fungal diversity

Our present study demonstrated the effects of tea-*Pleurotus* intercropping to soil fungal communities and properties. Here, we demonstrate an apparent difference in fungal community response based on intercropping period and practices, for example, it is evident that fungal richness in the 5 yr intercropping cultivations was significantly higher than the other three

Fig. 6. The redundancy analysis plot of soil fungal community composition and soil physicochemical properties: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 yr chemically fertilized monoculture plantations (M1). [Colour online.]



tea plantation cultivations (Fig. 2). However, fungal diversity is relatively higher in the 2 yr intercropping compared with other plantation intercropping treatments. Unsurprisingly, the 1 yr intercropping had the lowest number of OTUs and overall diversity indices. Our results indicate that the effects of the longer or continuous period of intercropping may have positive effects on fungal richness, while shorter intercropping may support the peak soil fungal diversity in tea plantations. This indicates that changes in fungal community structure in Tea-Pleurotus cultivation may vary over time and depend on the mode of intercropping. This further suggests the effects of intercropping are beneficial in long-term tea cultivation when fungal richness is taken into consideration. Our findings are similar to patterns observed by Song et al. (2021) demonstrating that continuous peach-*Morchella* intercropping improved soil structure while fungal diversity decreased. In the same study, the observed plant growth in intercropped treatments was enhanced by the improved soil fertility and physicochemical properties. Generally, in our study, we found fungal community significantly improved in fungal-intercropped tea plantations compared with those without intercropping. This result may be explained by the increased content of hydrolyzed N and available K in long-term intercropping, which is favorable in stimulating fungal growth in soils, which then contributes to overall soil processes (Vosnjak et al. 2021). Furthermore, previous findings from both experimental (Vosnjak et al. 2021) and natural grassland ecosystems (Li et al. 2019) reported similar patterns of results, showing that changes increase in N and P soil showed a positive relationship to the diversity of fungal communities in soil ecosystems.

Effect of tea-fungus intercropping on fungal community composition

A total of 3493 OTUs were obtained from four tea plantations represented by Ascomycota (44.7%), followed by Basidiomycota (17.7%) and Mortierellomycota (11.4%), while the other remaining phyla accounted for a much smaller proportion of other groups (Fig. 3A). Our findings conform with previous global soil sampling study, showing that the most abundant fungal phyla based on OTUs detection are represented by Ascomycota (48.7%) and Basidiomycota (41.8%), indicating that they are naturally abundant in most soil ecosystems (Tedersoo et al. 2014). In addition, studies on various ages and modes of tea cultivation showed that the most dominant groups are mainly Ascomycota (60%) and Basidiomycota (34%) (Wang et al., 2017). The dominant presence of Ascomycetes has beneficial roles in these systems as they contribute to increased degradation of decayed organic matter and degrade cellulose (Lim et al. 2010). The dominant phylum Basidiomycota in our samples may be an important factor to plant growth as it colonizes plant roots and aids plants the plants obtain nutrients, such as P from the soil (Lim et al. 2010). The other dominant is phylum Mortierellomycota and was highest in the 5 yr tea-*Pleurotus* intercropped tea plantation compared with the chemically fertilized plantation. This phylum is a common beneficial fungus to other soil microorganisms, not only promoting plant growth but also increasing the resistance level of plants (Ozimek and Hanaka 2021) and controlling root rot disease in plants (Whipps 2004).

The tea-*Pleurotus* intercropping in our study showed to support the relative abundance of the most important soil fungal phyla Mortierellomycota and Ascomycota, but not Basidiomycota. In addition, compared with the monoculture cropping tea plantations, the relative abundance of Ascomycota differed among three intercropping plantations and the relative abundance was the highest in the 2 yr intercropping, followed by the 1 yr intercropping, and the lowest in the 5 yr intercropping (Fig. 3A). Conversely, the trend in the abundance of *Mortierella* decreased with the decrease in intercropping years, and Mortierellomycota had a significant positive correlation with soil properties except for TK and AP in the 5 yr cropping. Likewise, *Mortierella* and *Fusarium* were the most dominant taxa in 5 yr cropping soils (Fig. 3A). Our results indicate that the dominance of *Fusarium* in subtropical tea plantations is consistent with the findings of Shen et al. (2015) and Wang et al. (2017), and they reported that *Fusarium* was the dominant group in the rhizosphere of banana and tea plantations. This may be explained by the increase in soil nutrients by intercropping that promotes optimal growth of *Fusarium*. In addition, *Archaeorhizomyces* is the second most dominant fungal group after intercropping cultivations. This taxon is chiefly found in the rhizosphere of plants, closely

Table 2. Mean values of physicochemical properties of soil under different tea plantations.

Properties of soil	I1	I2	I3	M1
TC ($g{\cdot}kg^{-1}$)	10.092 ± 0.672c	12.528 ± 0.722c	46.100 ± 1.374a	17.638 ± 1.338b
TN ($g{\cdot}kg^{-1}$)	1.018 ± 0.043d	1.285 ± 0.084c	3.4525 ± 0.055a	1.528 ± 0.052b
TP ($g{\cdot}kg^{-1}$)	0.188 ± 0.015c	0.253 ± 0.026b	0.523 ± 0.025a	0.253 ± 0.008b
TK ($g{\cdot}kg^{-1}$)	4.900 ± 0.103b	5.278 ± 0.272b	8.595 ± 0.049a	8.180 ± 1.249a
HyN ($mg{\cdot}kg^{-1}$)	115 ± 6.416c	220.750 ± 44.425b	321 ± 6.964a	153.250 ± 6.524 bc
AP ($mg{\cdot}kg^{-1}$)	4.058 ± 1.409b	22.200 ± 9.364a	15.598 ± 1.904ab	8.590 ± 1.785ab
AK ($mg{\cdot}kg^{-1}$)	143.500 ± 25.786b	169.500 ± 11.206b	484.250 ± 37.964a	192.750 ± 12.539b
OM ($g{\cdot}kg^{-1}$)	17.265 ± 1.320c	21.530 ± 1.175c	78.988 ± 3.215a	30.555 ± 2.193b

Note: 1 yr tea-fungus intercropping plantations (I1), 2 yr tea-fungus intercropping plantations (I2), 5 yr tea-fungus intercropping plantations (I3), and 5 -yr chemically fertilized monoculture plantations (M1). TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HyN, hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium; OM, organic matter. Values with different lowercase letters in the column (mean ± SD, $n = 4$) are significantly different at $P < 0.05$.

related to plant growth. All in all, our findings generally suggest that the intercropping of *P. ostreatus* if harnessed effectively can potentially improve the soil biota in tea plantations and may have important implications on plant growth, disease, and stress resistance of cultivated tea plants.

Diversity analysis using PCOA revealed that the fungal community composition of 1 yr intercropping and 2 yr intercropping had a slight difference in fungal community composition, compared with 5 yr intercropping and chemically fertilized plantation (Fig. 4). For example, the number of unique OTUs is highest in 5 yr intercropping compared with other intercropping years (Fig. 3). Therefore, the intercropping of *P. ostreatus* in tea cultivation practices offers long-term benefit to the soil ecosystems by increasing soil fertility but reducing the negative effects of monoculture plantation such as the reduction of soil biodiversity that comes with the increase of plantation age (Alekseeva et al. 2011; Wang et al. 2016; Hacisalihoglu et al. 2018).

Edaphic factors influencing fungal community structure

Soil chemical properties and fungal communities are closely related to each other. Soil fertilization is an important soil management method that improves plant growth and has significant effects on the structure of soil microbial communities (Treseder 2008; Zhang et al. 2016; Zhou et al. 2016). Growing edible fungi in the tea plantation require a large number of cultivation materials but offer a significant benefits in optimizing in tea plantations significantly increased the content of soil total N, available N and K (Han and Chen 2013) while another fungal taxon, *O. radicata* also significantly increased the content of available N, P, and K in tea plantations (Jing et al. 2018). In our current study, tea-*Pleurotus* intercropping increased soil organic matter, total and available N, which compensates for the high N requirements in tea plantations (Zhang et al. 2020; Zhang et al. 2020). The amount of soil total C, N, P and K, hydrolyzed N, available K, and organic matter showed an upward trend with the increase of tea-*Pleurotus* intercropping cultivation years, for example, soil properties of the soil in 5 yr intercropping were generally higher compared with other plantation (Table 3). Hence, continuous or longer tea-*Pleurotus* intercropping positively affects the quality of the soil compared with monoculture tea cultivations that reduce soil nutrition and fertility in the long-term planting cultivations (Arafat et al. 2017; Gu et al. 2019; Han et al. 2007; Li et al. 2016).

Table 3. Significance of fungi community in relation with the physicochemical properties of soil from different tea plantations.

Soil properties	RDA1	RDA2	r^2	P value
TC	0.953	0.302	0.735	0.002^{**}
TN	0.943	0.333	0.721	0.002^{**}
TP	0.918	0.397	0.560	0.008^{**}
TK	0.909	−0.417	0.618	0.004^{**}
HyN	0.854	0.520	0.521	0.007^{**}
AP	−0.015	0.999	0.060	0.655
AK	0.924	0.384	0.538	0.01^{**}
OM	0.956	0.293	0.740	0.002^{**}

Note: RDA1 and RDA2, coordinates of the tips of the vectors representing the explanatory variables; TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HyN, hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium; OM, organic matter. Values with $^{*}P < 0.05$, $^{**}P < 0.01$.

The results of the relationship between environmental factors show that C, N, P and K, hydrolyzed N, available K, and organic matter promote soil fungal community composition overall. Furthermore, soil fungal composition is promoted in the 5 yr tea-*Pleurotus* intercropping and may essentially influenced by total C, N, P, K, hydrolyzed N, available K, and organic matter. This trend between soil fungal taxa and properties sheds light on the increased presence of beneficial fungal taxa that may influence soil fertility and plant growth. For example, *Mortierella* is relatively abundant in the 5 yr intercropping plantation. This fungal taxa can transform and utilize nutrients and other organic matter in soil and promote soil fertility in agricultural landscapes (Tamayo-Vélez and Osorio 2018; Ozimek and Hanaka 2021). Nonetheless, the cultivation mode of tea-*Pleurotus* fungus intercropping may not entirely influence the overall observed relationship, other factors should be considered, which we did not explore in the current work, such as the water content of the fungal inoculants, soil texture, and pH value that may influence the physical and chemical properties of the soil and fungal communities (Song et al. 2021). These caveats in the present work warrant further investigation in the future.

Conclusions

Our findings in this present work provided baseline information on the potential of tea-*Pleurotus* intercropping to improve fungal communities and soil chemical properties in tea plantations. Tea is one of the most important global economic crop products, yet monoculture practices in this system pose environmental concerns in the long term, and alternative strategies to circumvent this is an important step-forward for sustainability in this agricultural system. Our findings in the present study revealed the effects of tea-*Pleurotus* intercropping on soil fungal communities in traditional Chinese tea plantations. Five years tea-*Pleurotus* intercropping significantly promoted the diversity of soil fungal community, while the 2 yr tea-*Pleurotus* intercropping is crucial for soil fungal community structure. The variation in soil fungal community is influenced by combined changes in soil properties and mode of tea-*Pleurotus* intercropping. Although the application of chemical compound fertilizer can still support soil fungal communities, the benefits to maintain soil natural biota are incomparable to tea-*Pleurotus* intercropping. In addition, long-term application or excessive application of chemical compound fertilizers has both positive by increasing microbial biomass (Geisseler and Scow 2014) and negative effects such as changes in the physical and chemical properties of the soil, such as soil acidification, and soil compaction, which enormously impacts soil microbial communities and its total environment (Beiying et al. 2010). Fungal intercropping and the conventional fertilizer application, if properly strategized, can potentially work in synergy to optimize tea cultivations. Our findings offer important baseline evidence in establishing more ecologically friendly tea cultivation in terms of effective soil conservation and management through tea-*Pleurotus* intercropping practices. Furthermore, *P. ostreatus* is a widely cultivated fungal taxa that are easy to grow in a wide suite of environmental conditions making its application cost-effective and highly available to many agroecosystems (Das and Mukherjee 2007).

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