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RESEARCH ARTICLE

INTERCROPPING TEA PLANTATIONS WITH SOYBEAN AND RAPESEED ENHANCES NITROGEN FIXATION THROUGH SHIFTS IN SOIL MICROBIAL COMMUNITIES

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KEYWORDS

intercropping, rapeseed, soil microbe, soybean, tea garden

HIGHLIGHTS

- Intercropping change soil bacterial communities in tea plantations.
- Intercropping increasing nitrogen cycling in the soils of tea plantations.

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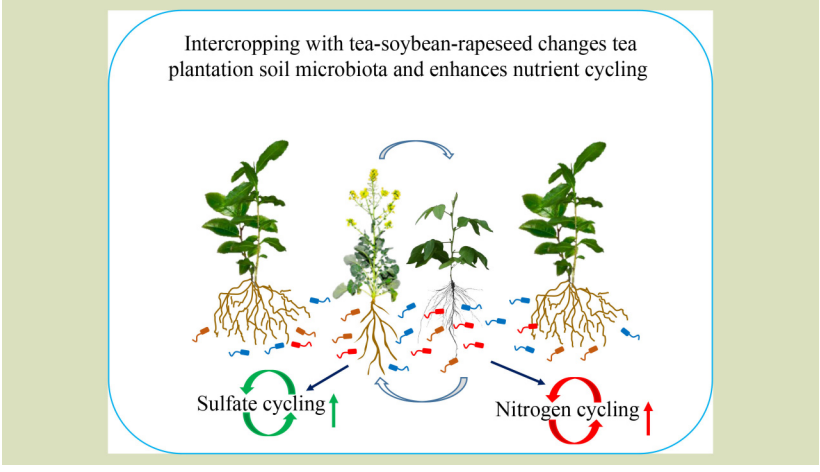
GRAPHICAL ABSTRACT



ABSTRACT

Intercropping with eco-friendly crops is a well-known strategy for improving agriculture sustainability with benefits throughout the soil community, though the range of crop impacts on soil microbiota and extent of feedbacks to crops remain largely unclear. This study evaluated the impacts of different intercropping systems on soil bacterial community composition, diversity, and potential functions in tea gardens. Intercropping systems were found to be significantly influenced soil microbiota. Within the three tested intercropping systems (tea-soybean, tea-rapeseed and tea-soybean-rapeseed), the tea-soybean-rapeseed intercropping system had the most dramatic influence on soil microbiota, with increases in richness accompanied by shifts in the structure of tea garden soil bacterial networks. Specifically, relative abundance of potentially beneficial bacteria associated with essential mineral nutrient cycling increased significantly in the tea-soybean-rapeseed intercropping system. In addition, soil microbial functions related to nutrient cycling functions were significantly enhanced. This was in accordance with increasing relative abundance of nitrogen cycling bacteria, including *Burkholderia* spp.

and *Rhodanobacter* spp. Based on these results, it is proposed that intercropping tea plantation with soybean and rapeseed may benefit soil microbiota, and thereby promises to be an important strategy for improving soil health in ecologically sound tea production systems.

1 INTRODUCTION

Tea cultivation originated in China and over centuries, tea consumption has spread globally to the point where tea has become the most popular beverage in the world[1,2]. Tea plants (*Camellia sinensis*) are now cultivated in more than 50 counties and consumed in at least 100 countries[3–5]. In China alone, an estimated 3.1 Mha of tea plantations yielded 2.78 Mt of tea in 2019[5].

One challenge often faced in the acidic soils common across tea-growing regions is the low bioavailability of nutrients, especially nitrogen and phosphorus[6,7]. Nitrogen, in turn is widely considered the most important mineral nutrient for tea plant growth and amino acid biosynthesis, an important component of tea quality[8]. To increase the economic returns of tea production, large amounts of chemical fertilizers have been applied to tea gardens, often improperly or in excess[9,10], which may lead to severe soil acidification and degradation, disturbance of the local soil microbial-ecology, water eutrophication and even detrimental impacts on tea quality[11–14]. Also, these problems can increase in severity as long as the implementation of alternative affordable and eco-friendly plantation management strategies is postponed[13].

Intercropping is an ancient agriculture practice in which complementary crops are grown between major crop species[15,16]. Published works have demonstrated conclusively that intercropping can be a key component of sustainable agriculture with benefits for crop yield and quality[17,18]. In addition, intercropping also influences the soil microbial communities, like increases in microbial diversity, carbon assimilation and nitrogen input[13,19,20]. At present, intercropping remains popular in small holders where it allows for increases in diversity of harvested products, as well as, reductions in amendments[21,22]. Leguminous plants are commonly used as intercropped plants for their nitrogen fixation capacities, which also allows them to be used as green manure[20,23,24].

Previous studies have outlined several eco-friendly practices designed to lower and optimize input applications on tea plantations[25,26], including incorporation of intercropping systems[27], such as planting soybean and rapeseed between rows of tea plants, which not only increases tea yields, reduces pesticide requirements, but also influences soil physical and chemical properties. The changes of soil properties usually cause shifts of soil microbial communities in the context of soil natural acidification[21,27,28]. However, little is known about the influence of eco-friendly practices (intercropping systems) on the community and functions of soil microbes in comparison to standard practices. In this study, we investigated and compared the influence of different intercropping systems on the structure, composition and potential functional capacities of soil microbiota in tea garden systems. The results suggest that continuous intercropping with soybean and rapeseed exerts the most influence of tested systems on the composition, structure and functional capacities of tea garden soil microbial communities, including notable increases in nitrogen fixation capacity.

2 MATERIALS AND METHODS

2.1 Plant materials and experiment site

The soybean cv. Huachun 6, rapeseed cv. Zhongshuang 11 and 30-year-old plants of the tea cv. Rougui were used in this study. The experiment was sited at Yan Zike, Wuyi mountain, Nanping city (27.66° N, 117.93° E).

2.2 Intercropping cultivation

The experiment included three intercropping systems, namely tea-soybean (IS), tea-rapeseed (IR) tea-soybean-rapeseed (ISR), and control tea rows with no intercropping (IC). A schematic diagram of the tested intercropping systems and soil sample collection is given in Fig. 1. Specifically, soybean seeds were sown in the middle of the rows between tea plants at the density of two seeds per 30 cm in June 2018 (Fig. 1(a)). Before sowing, soybean seeds were inoculated with rhizobia as previously described[29]. Rapeseed seeds were spread in between rows of tea plants in November 2018, and rapeseed plants were cut at the ground as green mature after flowering as

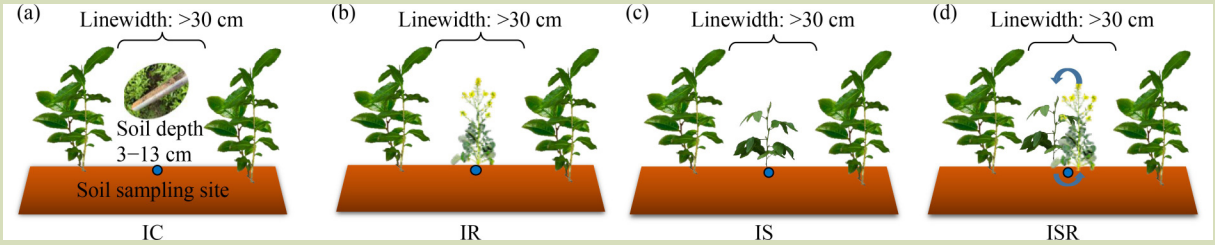


Fig. 1 Experimental design of soybean and rapeseed intercropping in this study. Diagram of tea rows and soil sampling sites in the tea garden and the intercropping systems (tea-control, tea-soybean, tea-rapeseed and tea-rapeseed-soybean) used in this study in the tea garden. The distance between tea rows are usually greater than 30 cm. with the soil sampling locations central between the tea rows. (a) IC, tea-control; (b) IR, tea-rapeseed; (c) IS, tea-soybean; (d) ISR, tea-rapeseed-soybean intercropping.

described by Liang et al.[27]. For IS plots, no crops were planted after soybean; while for IR plots, rapeseed was sown between tea rows with no prior soybean plantings. In ISR plots, rapeseed was planted between tea rows after soybean maturation, thereby making it a rotation of soybean and rapeseed. The three intercropping systems and control plots were replicated in five randomized blocks. Each treatment plot in every block contained five rows of tea plants at least 10 m in length.

2.3 Sampling methods for soil microbes

Soil samples were collected centrally between tea rows about 1 month after cutting rapeseed plants as green manure. Soils were taken from the depth of 3–13 cm below the surface by removing the top 3 cm of soil according to Zhong et al.[29]. Five soil columns were collected from each plot and mixed together as a sample, and two samples were taken for each replication of each treatment. In total, 10 independent soil samples representing 50 soil columns were collected for each treatment and stored at −80 °C prior to total soil DNA extraction.

2.4 DNA extraction and sequencing

Total DNA was extracted from 0.5 g of soil using the PowerSoil DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Concentrations of extracted DNA were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 regions of 16S rDNA were amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)[30]. PCR was conducted using the Phusion High-Fidelity PCR Master Mix with GC Buffer (NEB, Ipswich, MA, USA) in a thermocycler PCR system (GeneAmp 9700, ABI, USA). Amplified PCR products were detected on 2% agarose gels and target bands were purified using the QIAquick Gel Extraction Kit (Qiagen, Dusseldorf, Germany). High throughput sequencing was performed by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) on an Illumina Miseq platform (Illumina, San Diego, CA, USA).

2.5 16S amplicon sequence analysis

The QIIME2 (Quantitative Insights Into Microbial Ecology 2) Linux analysis platform was used for the raw next-generation high throughput sequencing data derived from the Illumina Miseq platform[31]. Specifically, q2-demux and q2-cutadapt trim-pairs were used to remove the barcodes and linkers sequence[32]. Then the sequences were further subjected to the paired end reads merging with vsearch software[33]. Sequences were then subjected to the quality control with quality-filter and deblur plugins in QIIME 2 (quality score > 25) and then denoising[34]. The OTUs (operational taxonomic units) were clustered with 97% sequence similarity and annotated using SILVA database with q2-feature-classifier plugin[35,36]. Alpha diversity, unweighted/weighted-UniFrac distance matrix and Bray-curtis distance matrix were calculated with QIIME2. Analysis of similarities (ANOSIM) was performed based on Bray-Curtis distance matrix with permutation test. The number for permutation test was 999. Constrained principal component analysis (CPCoA) were performed with ANOVA-like permutation analysis using "Vegan" package (version 2.4.0) in R (version 4.3.1). LEfSe analysis was performed using the online program (huttenhower.sph.harvard.edu/galaxy) with a normalized OTU table. Kruskal–Wallis rank sum tests with an alpha value of 0.05 and a threshold of 2 or 3 were used to identify biomarkers. CPCoA was used to calculate the contributions of intercropping systems. Functional predications in mineral nutrient cycling were carried out using FAPROTAX database[37].

2.6 Statistical analysis

Means and stand error values were calculated using SPSS software version 19[38]. Permutation testing was used to analyze differences at the community level in ANOSIM. Duncan's multiple comparison testing was used to separate means among significant treatments. Illustrations were constructed in GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA) and R (version 4.30).

3 RESULTS

The intercropping plants were cultivated in the middle space between tea rows. Soil chemical property analysis of tea garden soil under different treatments showed that the soil pH, OM (organic matter), AN (alkali-hydrolyzable nitrogen), AP (available phosphorus) and AK (available potassium) were changed at various degrees by the intercropping treatment (Table 1). Soil bacterial communities were assessed through high throughput 16s rDNA amplicon sequencing. Rarefaction curves showed that the sequencing depth was enough to cover most of the bacteria in the samples (Fig. 2(a)). In addition, Chao1 index and Shannon index indicated that intercropping influenced the richness but not the community diversity of soil bacteria (Fig. 2(b,c)). Specifically, bacteria communities in IR and ISR were significantly increased by comparison with IC. Overall, intercropping did not change the diversity of bacterial community, but did increase the richness of bacteria in the soil of IR and ISR in the tea garden.

In addition, distinct bacterial community structures were discernible among the studied intercropping systems, with intercropping treatment accounting for 12% of variance and significantly impacting bacterial communities based on unweighted UniFrac distance by CPCoA analysis ($P < 0.001$) (Fig. 3(a)). In addition, CPCoA based on the weighted UniFrac showed similar results, with IC overlapped with IR treatment (Fig. 3(b)). More specifically, IS, IR and ISR treatments all significantly influenced the structure of bacterial communities in comparison with the IC plots (Fig. 3(a)). In addition, ISR had a greater influence ($R = 0.308$; $P = 0.001$) on the bacterial community than either IS ($R = 0.181$; $P = 0.007$) or IR ($R = 0.166$; $P = 0.007$). Taken together, these results indicated that different intercropping systems had different significant

Table 1 Basic soil chemical properties under four intercropping treatments

Group	pH	Organic matter (OM) ($g \cdot kg^{-1}$)	Alkali-hydrolyzable nitrogen (AN) ($mg \cdot kg^{-1}$)	Available phosphorus(AP) ($mg \cdot kg^{-1}$)	Available potassium (AK) ($mg \cdot kg^{-1}$)
IC	4.46 ± 0.06ab	18.0 ± 1.8b	71.1 ± 9.4a	52.9 ± 16.9a	130 ± 10.3a
IS	4.60 ± 0.04a	24.2 ± 1.6b	62.7 ± 4.4ab	30.6 ± 6.5a	103 ± 8.0b
IR	4.43 ± 0.03b	22.3 ± 1.1b	70.6 ± 4.0a	44.3 ± 9.9a	133 ± 6.0a
ISR	4.59 ± 0.05a	31.5 ± 2.5a	52.4 ± 3.3b	32.5 ± 12.6a	100 ± 12.1b

Note: Data represent means ± S.D.; Different letters indicate significant differences among different treatments and different groups contain with same letter indicate non-significant differences among different treatments in Duncan's multiple range comparison test.

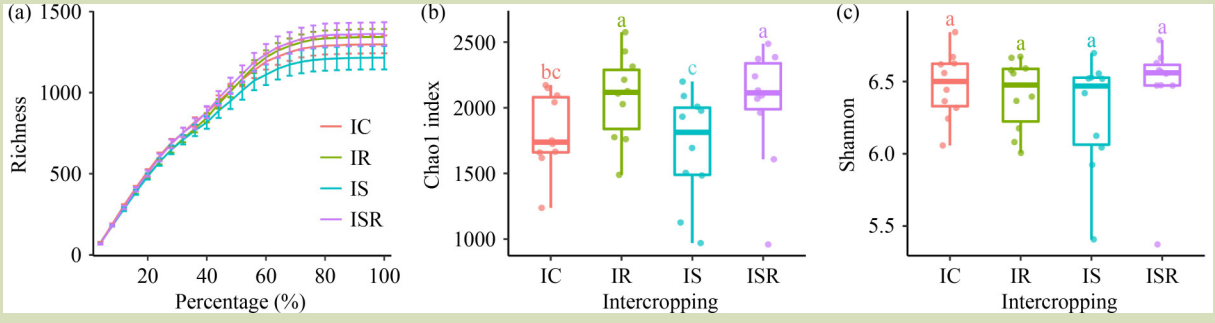


Fig. 2 Richness and diversity of soil bacterial communities in tea garden under four intercropping systems. (a) Rarefied fraction curves of bacterial community richness in each treatment. (b,c) Comparison of bacterial communities associated with each intercropping treatment using the Chao1 index (b) and Shannon index (c). Median of treatments with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$). IC, tea-control; IS, tea-soybean; IR, tea-rapeseed; ISR, tea-rapeseed-soybean intercropping.

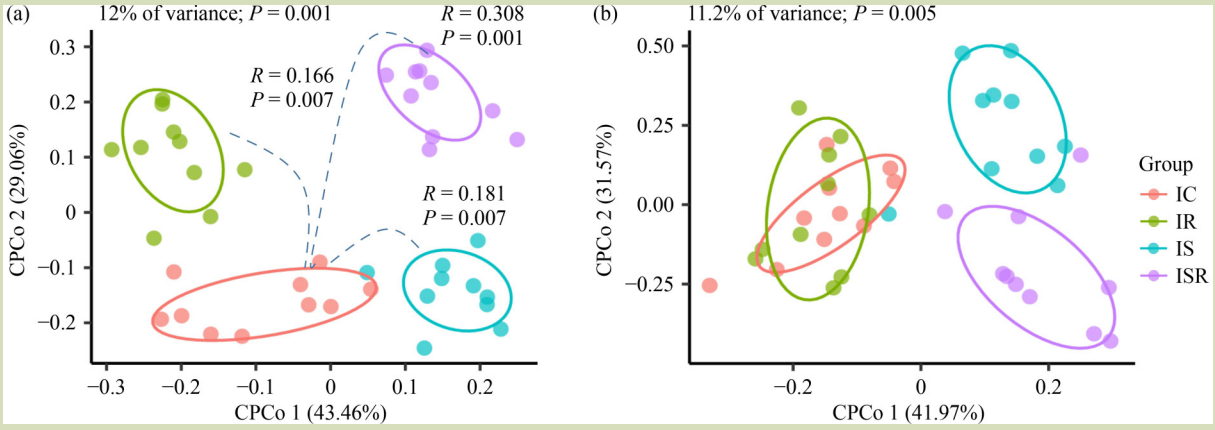


Fig. 3 Influence of four intercropping systems to the structure of soil bacterial communities. Constrained PCoA of bacterial communities in tea garden soils based on distance matrix of unweighted UniFrac (a) and weighted UniFrac (b) using the *anova.acc()* function in R "Vegan" package (version 2.4.0). Comparisons of soil bacterial communities of four intercropping systems with control plots using ANOSIM analysis. The *P*-value was calculated by permutation testing (n = 999).

impacts to the structure of bacterial communities in soils.

Overall, bacterial composition analysis at the phylum taxonomic level indicated that Proteobacteria, Chlorobacteria, Bacteroidetes, Acidobacteria and Actinobacteria were the dominant bacterial phyla present in the studied tea garden soils, with no significant differences observed between intercropping systems at this level (Fig. 4(a)). This indicated that soil bacterial communities were relatively stable at the phylum taxonomic level under different cropping systems. However, a Venn diagram showed that specific soil bacteria were influenced by the intercropping. There were147 OTU, 250 OTU, 163 OTU and 240 OUT were specifically detected in the IC, IR, IS and ISR, and 3367 OTUs presented as common OTUs in all the treatment (Fig. 4(b)).

Analysis of co-occurrence within bacterial communities revealed that intercropping with soybean, IR or ISR increased the connectivity of the co-occurrence networks, with clustered bacterial modules forming within the networks. The node number and connectivity number for IC, IS, IR and ISR treatments were 172 and 917, 189 and 1191. 186 and 1161, and 187 and 1338 (Fig. 5), respectively. In addition, the average degree of the networks for the IC, IR IS and ISR were 10.6, 12.5, 12.6 and 14.3. Module analysis showed there are two, three, four and four modules present in the networks of IC, IR, IS and ISR. In addition, the average weight degree for the networks of IC, IR, IS and ISR were 4.77, 8.87, 6.79 and 10.9 (Fig. 5). Taken

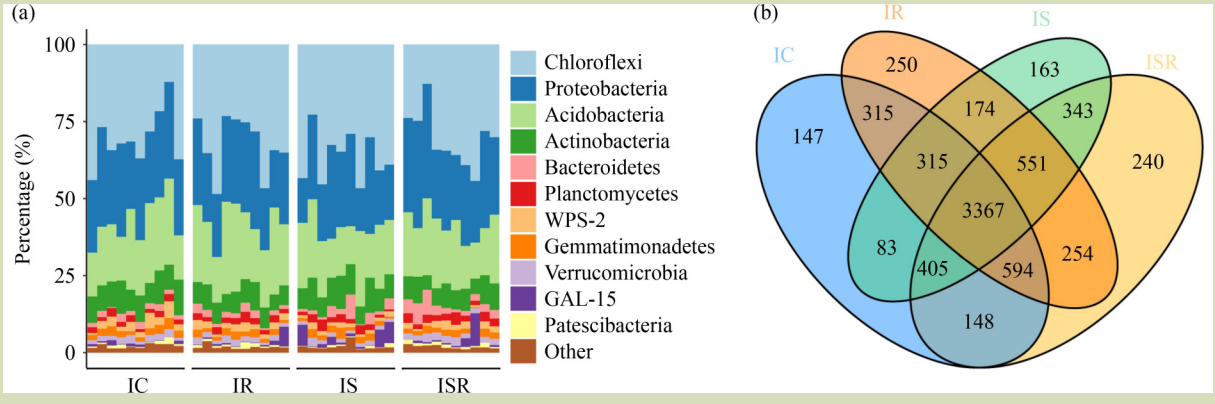


Fig. 4 Influence of four intercropping systems to the composition of soil bacterial communities. (a) Composition of bacterial communities at phylum taxonomic level in a tea garden under four intercropping systems. (b) Venn diagram of overlapping OTUs and specific OTUs in tea garden soil under four intercropping systems.

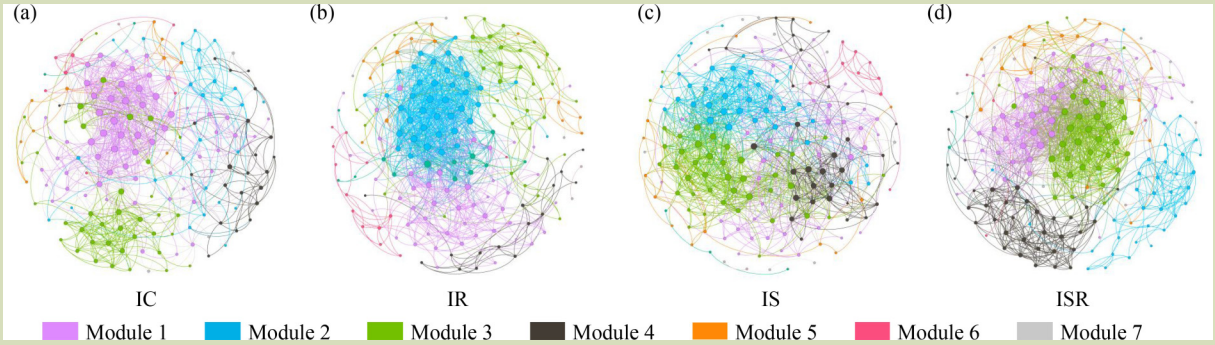


Fig. 5 Intercropping drives the establishment of distinct microbial networks. Comparisons of bacterial co-occurrence networks in the soils of a tea garden subjected to four intercropping systems. Co-occurrence networks were constructed based on correlation analysis of the relative abundance of microbial OTUs across replicates and intercropping treatments. The size of the nodes is proportional to the number of connections to other nodes in the network. The color of nodes indicates the module of the microbes as shown in the key. Connections between nodes represent significant correlations ($P < 0.05$ and $r < 0.70$ in Spearman's rank correlation test). Hub microbes in each co-occurrence network are indicated by darker nodes. (a) IC, tea-control; (b) IR, tea-rapeseed; (c) IS, tea-soybean; (d) ISR, tea-rapeseed-soybean intercropping.

together, these results showed that the complexity of the networks in the intercropping were higher than the control and intercropping influence the bacterial co-occurrence network of soil bacteria in the soil of tea garden.

More detailed analysis was conducted with microbes that were significantly influenced by intercropping (Fig. 6). Compared to relative abundances in control plots, Ktedonobacterales, Chloracidobacteria, Gemmatimonadales and Microbacteriacea all increased significantly in relative abundance with IS (Fig. 5(a)), while the relative abundances of *Rhodanobacter*, Streptphyta and Holophagae significantly increased with IR (Fig. 6(b)), and *Burkholderia*, Xanthomonadaceae, Sphingomonadaceae, Micrococcaceae, Holophagae and Comamonadaceae and other bacterial belonging to Proteobacteria all became significantly more abundant with ISR (Fig. 6(c)). Taken together, these results indicated that intercropping with different plants or using different intercropping strategy significantly influenced the relative abundance of specific microbes in soils.

To further investigate the functional changes along with the shifting of soil bacteria due to different intercropping systems, we analyzed the potential functions of soil bacteria under different treatments. We mainly focused on the nutrient element cycling genes. The results revealed that functions related to nitrogen cycling were changed to varying degrees (Fig. 7(a–f)). Especially, the abundance of nitrogen fixation genes, nitrification genes, nitrate reduction genes and nitrate denitrification genes were all significantly increased in the ISR group, and nitrite oxidation genes were significantly increased in the IR treatment. In addition, sulfate oxidation genes were also significantly increased in the ISR treatment and no significantly changes were observed in iron respiration (Fig. 7(g,h)). In combination, these results implied that intercropping not only shifted the soil bacterial community but also promoted the potential functions related to soil nutrient elements cycling.

4 DISCUSSION

Intercropping has long been recognized for multiple beneficial impacts on agroecosystems, including increasing yields of major crops, improving soil biodiversity and health, and simultaneously reducing damage caused by plant pests and pathogens[16,18,20]. Yet, knowledge on specific effects of intercropping on components of soil microbial-ecology remains sparse. In this study, we found that different intercropping systems have various effects on the structure, composition and potential functions of bacterial communities in tea garden soils.

Leguminous plants are commonly chosen for intercropping systems designed to increase biological nitrogen fixation capacity, improve soil fertility, and provide fixed nitrogen to neighboring and subsequent crops[39–41]. This study also included rapeseed as a potential contributor of phosphorus released from fixed soil phosphates as previously reported for Brassicaceae plants[42]. Continuous intercropping with soybean and rapeseed might, therefore, be reasonably paired in intercropping systems designed to benefit partner crops

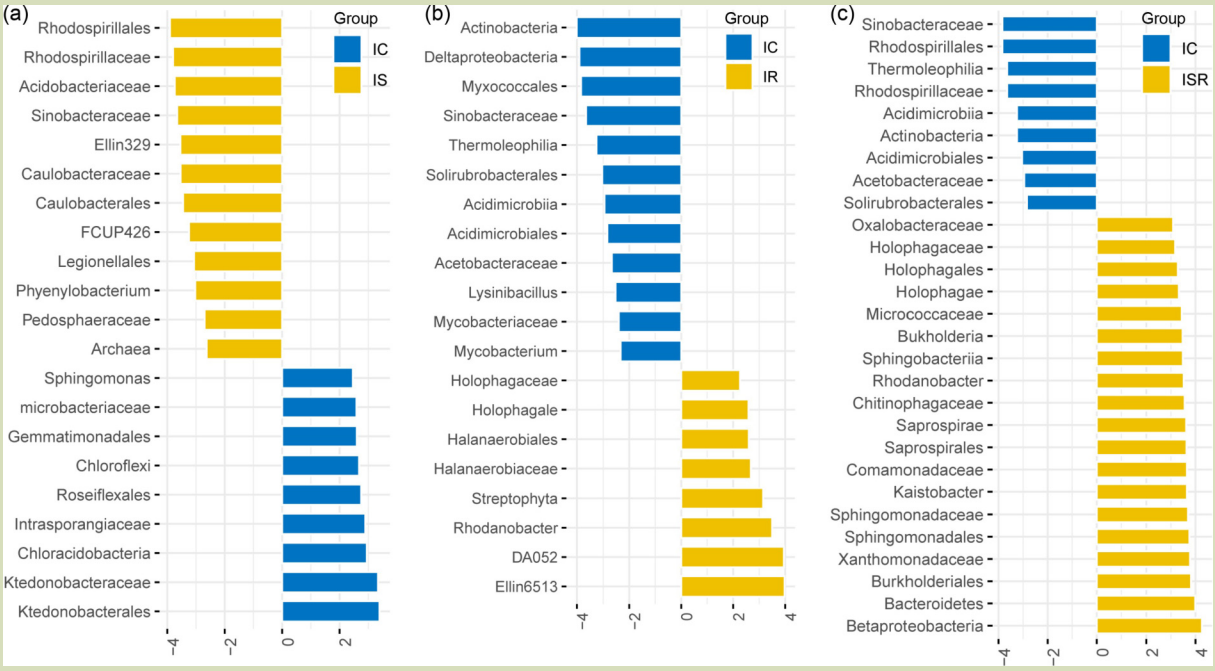


Fig. 6 Biomarkers for four intercropping system. Comparisons of bacteria identified as significantly different between the four intercropping systems tested using LEfSe analysis and presented as LDA scores of significant biomarker bacteria between IS and IC (a), IR and IC (b) and ISR and control (c) treatments. LDA scores are shown as horizontal bars for biomarker bacteria returned as significant in the Kruskal–Wallis rank sum test at $P < 0.05$. LDA: linear discriminant analysis; IC: tea-control; IS: tea-soybean; IR: tea-rapeseed; ISR: tea-rapeseed-soybean intercropping.

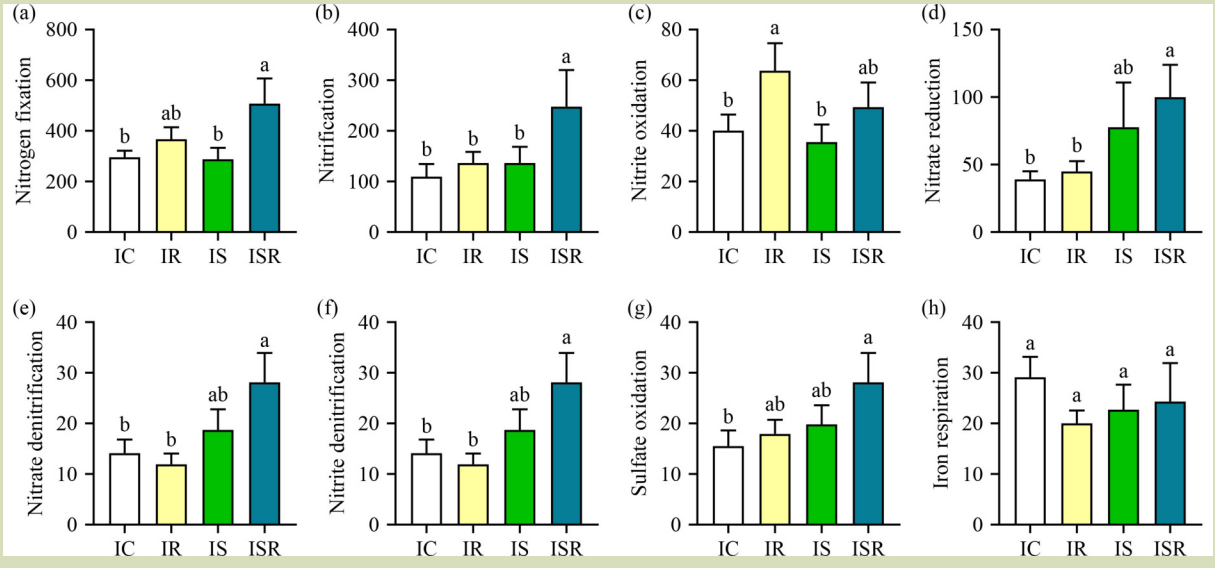


Fig. 7 Functional change of soil microbes for four intercropping patterns. Comparisons of potential nutrient cycling genes between different cropping system. Counts of function genes relative to nitrogen fixation (a), nitrification (b), nitrite oxidation (c), nitrate reduction (d), nitrate denitrification (e), nitrite denitrification (f), sulfate oxidation (g), and iron respiration (h) between different intercropping. Means with the same letter are not significantly different (Duncan's multiple range comparison tests, $P < 0.05$). IC: tea-control; IR: tea-rapeseed; IS: tea-soybean; ISR: tea-rapeseed-soybean intercropping.

through simultaneous increases in the bioavailability of both soil nitrogen and phosphate[6]. Our results showed that soybean-rapeseed and rapeseed intercropping significantly increased the richness of soil bacterial communities relative to control plots, while soybean intercropping had negligible impacts on overall soil community richness and no significantly changes were observed to the bacterial diversity (Fig. 2(b,c)). Field conditions and schedules that led to delays between soybean harvesting and soil sampling might have obscured impacts of intercropping with soybean alone. In addition, the AN content was lowest in the ISR treatment with the highest SOM contents, we postulated that this might be due to the higher bacterial richness in the ISR and the mineralized nitrogen might be absorbed by bacteria or uptake by the tea plants.

Among the microbes that were significantly influenced in IS plots, Ktedonobacteria has been reported to have an antinomycete-like morphology and belongs to the Chloroflexota[43,44], which have previously been isolated from black locust wood and paddy soil where they contribute a capacity to degrade cellulose and might be involved in mineralization of organic matter[43,45]. Kouleothrixaceae has been reported as a tightly associated keystone taxon in the rhizosphere of *Cistanthe longiscapa*[46], while Chloracidobacteria have been identified as dominant phyla in the rhizosphere of plants where functions remain largely unknown[47,48]. Microbacteriaceae are reported as plant associates that might be involved in attenuating negative impacts of biotic stress[49–51].

Intercropping rapeseed alone promoted *Rhodanobacter* spp., which have previously been reported as acid tolerant inhabitants of soils high in organic matter and the rhizospheres of soybean, tomato and ginseng[52–55]. *Rhodanobacter* populations have also been documented as antagonistic against the root rot fungal pathogen *Fusarium solani*[53], and might participate in nitrogen cycling[56–58]. Streptophyta has been reported as involved in phosphate metabolism where it promotes the release of phosphate and accumulation polyphosphate[59]. Halanaerobiales for its part might be involved K metabolism[60], while Holophagae members belonging to the Acidobacteria phylum have typically been specifically enriched in plant rhizospheres where they might be involved in sulfur metabolism[61–63]. However, the changes of soil microbial composition in soils were due to the directed influence of intercropping plants or due to the changes of soil chemical properties need further investigation (Table 1, Fig. 6)[64]. In combination, these results from intercropping with rapeseed strongly suggests that this partner might significantly increase mineral nutrient cycling and bioavailability.

Beyond intercropping with soybean or rapeseed alone, our results demonstrate that intercropping tea with a soybean-rapeseed rotation had the most dramatic influence on bacterial richness (Fig. 2(a,b)). Also, the structure of ISR bacterial communities varied not only in comparison with IC soil bacterial communities, but also in comparisons with the individual intercropped systems (Fig. 3). Overall, 240 OTU were specifically promoted only in the ISR treatment, but not in either IS or IR plots (Fig. 6). Among OTUs specifically enriched in ISR plots, Comamonadaceae was previously reported as involved in sulfur transformations[65], while Chintinophagaceae has been reported as a contributor to plant health through antagonism to fungal pathogens[66,67]. These ISR specific OTUs might complement others that are also enriched in IS or IR, such as *Rhodanobacter*, which is also enriched in IS plots (Fig. 6(a)). As a result, ISR treatments might significantly increase beneficial impacts on intercropped systems over intercropping with soybean or rapeseed alone through effects on the abundance of microbes contributing to nutrient cycling and plant defenses[68].

Importantly, functional predictions generated using FAPROTAX suggest that genes involved in nitrogen cycling such as nitrogen fixation, nitrification, nitrate reduction, nitrate denitrification and nitrite denitrification were all significantly higher in ISR than IC treatments (Fig. 7(a–f)). In addition, functions related to sulfate oxidation was also enhanced in ISR compared with IC treatment (Fig. 7(g)). These results suggest that intercropping with a soybean-rapeseed rotation may also influence the functions of bacteria along with changing of bacterial composition in soils and promote the soil nutrient cycling. This might due to feedback cycles promoted by the microbes that were significantly increased in the ISR treatment. For example, *Burkholderia* was enriched in ISR plots and is mostly identified as a plant growth promoting bacteria that usually carries the *nifH* gene[69–72]. Another bacterial clade enriched in the ISR treatment, *Kaistobacter*, has previously been enriched in the rhizospheres of healthy plants[73] where it too has been associated with plant growth promotion[74,75].

Variation in the relative abundance of specific OTUs among intercropping treatments was accompanied by distinct patterns in the soil bacterial community co-occurrence networks for each intercropping system (Fig. 5). Notably, each treatment produced clusters of co-occurring bacterial modules, though connectivity and module analysis were considerably higher in intercropping treatments than in control plots (Fig. 5). These results indicate that intercropping increases the potential interaction networks of soil bacteria, and also indicates that intercropping might confer beneficial impacts on soil health

and crop productivity[76]. Given the relative stability of communities observed at the phylum level (Fig. 4(a)), it could be useful to observe in future experiments whether distinctive elements of co-occurrence networks might be predictably stable over time.

5 CONCLUSIONS

This work revealed that intercropping with soybean, rapeseed and a soybean-rapeseed rotation have various degrees of influence on the composition of tea garden soil bacterial communities. Significant enhancement in relative abundances were observed for numerous potentially beneficial microbes, such as bacteria involved in accelerating nutrient cycling, such as nitrogen cycling, sulfate oxidation. Overall, these results suggest that intercropping soybean and rapeseed with tea plants might be a promising strategy for maintaining soil health and promoting sustainable production in tea gardens.

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Compliance with ethics guidelines

Yongjia Zhong, Lini Liang, Ruineng Xu, Hanyu Xu, Lili Sun, and Hong Liao declare that they have no conflicts of interest or financial conflicts to disclose. Sequencing raw data was deposited in the NCBI database under the accession number PRJNA788975. This article does not contain any studies with human or animal subjects performed by any of the authors.

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