Changes in Soil Microbial Community Structure and Functional Diversity in the Rhizosphere Surrounding Tea and Soybean

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ABSTRACT

Field trial was conducted to evaluate the effects of different planting patterns (tea monocropping, tea and soybean intercropping, soybean monocropping) on microbial community structure and microbial functional diversity using Biolog technique. Results showed that intercropping treatment exhibited higher average well color development (AWCD), diversity indices and community functional diversity as compared with monocropping. The microbial utilization of 6 types of carbon source indicated some differences. Principal component analysis and cluster analysis demonstrated that intercropping treatment significantly changed the functional diversity of the soil microbial community, mainly depending on carbohydrates and carboxylic acids. Our findings suggested that soil microbial metabolic activities and functional diversity were significantly changed by tea-soybean intercropping.

Keywords: *Intercropping, microbial functional diversity, rhizosphere, soybean, tea*

INTRODUCTION

Tea (*Camellia sinensis (L) O. Kuntze*) is one of the most popular beverages in the world due to its health benefits that have been investigated especially in cancer prevention due to the presence of polyphenolic substances. (Saravanakumar *et al.,* 2006; Yang *et al.,* 2007; Khokhar and Magnusdottir, 2002), Mainly tea is classified into three main types namely, black tea, green tea and oolong tea according to its production process (Takeo, 1992). It is also a major cash crop in many developing countries, including China, India and Sri Lanka. Currently, China is the largest producer of tea, which accounts for 74.8% of global area and constitutes 41.6% of world production, and the total area under tea plantation is 2.74 million hectares (ITC, 2015).

Soil is an important substance for the growth of the tea plant as well as the absorption of nutrients and therefore, only rich soil can produce high quality tea (Lin *et al.,* 2012). In the soil-plant ecosystem, the soil microorganisms are actively involved in the material and energy cycle of the ecosystem as one of the most active and decisive components (Doran and Zeiss, 2000), affected by a range of factors, such as planting patterns, soil type and climatic conditions. Planting patterns are one of the most important factors. Intercropping, as the essence of traditional agriculture, has been widely spread and applied in agricultural production. Compared to monocropping, numerous studies have shown that intercropping could increase efficiency of utilization of natural resources (Gao *et al.,* 2009; He *et al.,* 2013; Rivest *et al.,* 2010), reduce disease, insect and weed (Hummel *et al.,* 2009; Workayehu and Wortmann, 2011; Abdel-Monaima and Abo-Elyousr, 2012), boost crop yield (Songa *et al.,* 2007; Nataraj *et al.,* 2010;

Mao *et al.,* 2012) and enhance soil microbial diversity (Hinsinger *et al.,* 2011; Bainard *et al.,* 2011). Therefore, the fact that how to achieve high quality and yield of tea and sustainable development by ecological regulation attract urgent attention.

As a traditional planting model, tea intercrops with different crops which could increase soil organic matter content and nutrition, improve the micro-climate of tea garden (Sun *et al.,* 2011; Zhang *et al.,* 2014), inhibit weeds and pests, maintain tea garden ecological balance (Kamunya *et al.,* 2008; Ye *et al.,* 2016), promote the tea growth and improve the tea quality and yield (Bore, 2005; Sedaghathoor and Janatpoor, 2012). Previous studies were concentrated primarily on the yield, quality, soil fertility and natural biological diversity, but changes in soil microbial community were not well understood in the tea intercropping system. Therefore, a field trial was conducted to explore whether intercropping improves the soil microbial community structure and function diversity, and provides a further scientific basis for the high yield and quality in the tea intercropping system.

MATERIALS AND METHODS

Study site: Field experiments were conducted in a tea garden (10 years old) of Guangxi South Subtropical Agricultural Science Research Institute at Longzhou, Chongzuo, Guangxi province in Southwest China (22°21′N, 106°46′E). This region has a typical subtropical monsoon climate with an average annual precipitation of 1273.6 mm, an average annual temperature of 22°C , an altitude of 125 m and an annual sunshine of 1251 h. The soils are acid and red, and its initial properties were as follows: pH 5.86±0.21; soil organic matter 13.17±2.55 g kg^{-1}; available nitrogen 128.44±2.64 mg kg^{-1}; available phosphorus 30.68±2.43 mg kg^{-1}; and available potassium 174.75±3.03 mg kg^{-1}.

Experiment design:

Field trial was conducted with three planting treatments which consisted of tea monocropping, tea and soybean intercropping, soybean monocropping and three replications, on a total of 9 plots in a random block design, and the plot area was 24 m^2. For intercropping, the planting ratios of tea and soybean were 2:2, i.e. every 2 rows tea intercropped with 2 rows soybean per plot. Tea were planted with a row width of 0.4 m and a plant spacing of 0.30 m, and soybean with a row spacing of 0.3 m and a plant spacing of 0.15 m. The fertilizers were applied according to the local custom, and nitrogen fertilizers rates of tea were applied 3 times a year, as at 40%, 30% and 30%, and that of soybean were applied two times at rates of 60%, 40%. Phosphorus fertilizer (P$_2$O$_5$), Potassium fertilizer (K$_2$O) and organic resources were applied as base fertilizers. The fertilizer rates were same in each plot.

Soil sampling

The samplings took place at the beginning of July 2016. First, plant roots were taken off from the soil, shaken off the loose soil and then were brushed off for any remaining soil that was strongly adhered to the roots as rhizosphere soil. 4 plants per monocropping plot and 4 per intercropping plot were randomly selected and then the rhizosphere soils of 4 plants were mixed in one sample. Part of the soil was stored at 4 °C until being used for the microcosm experiment as described below, another part was air-dried, ground and passed through 1-mm and 2-mm mesh sieves for chemical analysis.

Measurements

Biolog Ecoplates (Biolog Inc., Hayward, CA, USA) were used to determine the soil microbial functional diversity based on the utilization of 31 carbon substrates (Garland and Mills, 1991). Fresh soils (10 g) were extracted by shaking for 30 min at 200 rpm with 90 ml 0.85% NaCl. Ten-fold dilutions were performed until the desired

(10^{-3}) dilution was reached. An aliquot (150 ul) of the diluted suspension was placed in each well of the Biolog Ecoplate using a multi-channel repetitive-dispensing pipette. The plates were incubated at 28°C, and the absorbance at 590 nm was recorded at 24 h intervals for 10 days using the reader incorporated into the Biolog GEN III Micro Station TM (USA). Three replicates per treatment and sampling time were performed. The readings at 120 h were used for the statistical analysis.

The overall rate of substrate utilization by microorganisms was measured by calculating the Average Well Color Development (AWCD) for each plate;

$$AWCD = \sum (C_i - R_i)/31$$

where C_i is the OD in each carbon source well and R_i is the OD of control well. The microbial community diversity was calculated by the Shannon index;

$$H = -\sum P_i (\ln P_i)$$

where P_i is calculated by subtracting the control from each substrate absorbance and then dividing this value by the total color change recorded for all 31 substrates, $P_i = (C_i - R_i)/\sum (C_i - R_i)$. The evenness was calculated as $E = H/\ln [richness (S)]$, where the richness (S) referred to the number of substrates utilized.

Statistical analysis

The carbon source utilization data were subjected to Principal Component Analysis (PCA) and Cluster Analysis (CA) using Microsoft Excel 2010 with Multibase. The microbial parameters for the different treatments were analyzed by Fisher's Least Significant Difference (LST) test at a significance level of 0.05 after verifying the significance by analysis of variance (ANOVA) using SPSS 19.0 software.

RESULTS

Changes in average well color development (AWCD)

The AWCD used to assess the utilization of overall carbon sources, is an important indicator that reflects the biological activity of soil microorganisms (Diosma *et al.,* 2006). General shifts of the AWCD in different treated soils and changes within the incubation period were shown in Figure 01-A. The total utilization of different carbon sources by soil microorganisms appeared increasing tendency with cultural time, but that was different in different planting patterns. The changes of AWCD values were not obvious within 24h, and then increased rapidly until it tended to stabilize. During the whole culture period, the AWCD values were generally higher in intercropping treatment than that observed under monocropping treatment. Intercropping soybean exhibited the highest AWCD values, whilst the lowest AWCD values were found in monocropping tea.

As indicated in Figure 01-B, on the culture time of 120h, intercropping increased the AWCD values of tea and soybean by 11.52%, 12.99% respectively when compared with monocropping, and with a significant difference between monocropping and intercropping soybeans. Moreover, the AWCD values of soybean were greater than that found in tea. The above analysis suggested that tea and soybean intercropping can promote the utilization of carbon sources by soil microorganisms to increase microbial metabolic activity.
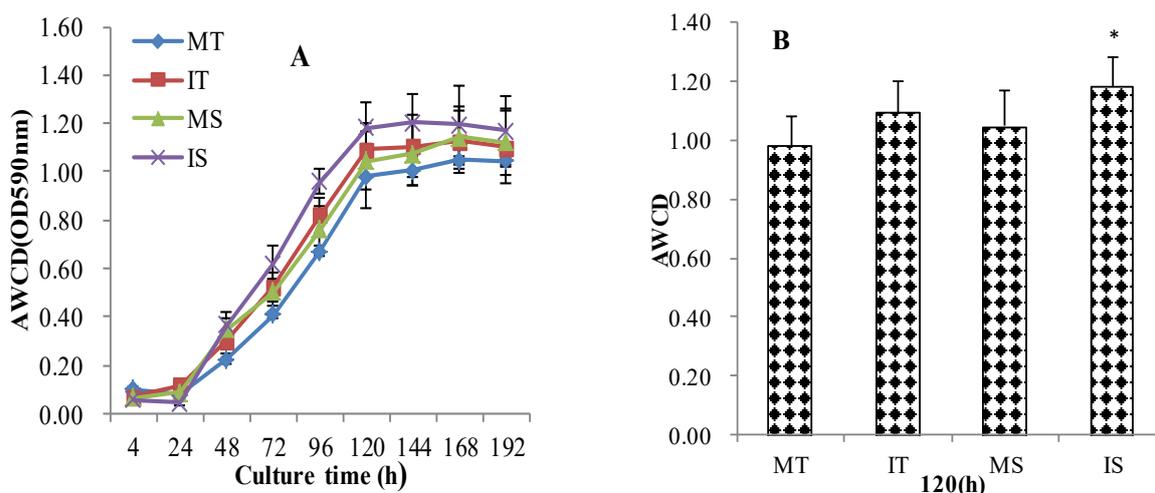
Figure 01: Effects of intercropping on average well color development (AWCD) of 31 carbon sources

Note: MT: Monocropping tea; IT: Intercropping tea; MS: Monocropping soybean; IS: Intercropping soybean. * mean significant difference between monocropping and intercropping pattern (P<0.05). The same below.

Differences in utilizing six types of carbon source by soil microorganisms

Soil microbial activity reflects the total changes of microbial community, and fails to show the detailed information about metabolism, so, study the differences in utilization of different carbon source contribute to more fully understanding the characteristics of microbial community metabolic function (Weber *et al.,* 2007). As shown in Figure 02, the utilization of carbohydrates, carboxylic acids, amino acids and amides by soil microorganisms in the rhizosphere surrounding tea and soybean were stronger, the polymers and phenolic acids were weaker in monocropping and intercropping treatments.

As shown in Figure 03, the microbial utilization of 6 types of carbon source in the rhizosphere surrounding tea and soybean were affected by intercropping, but they had some differences. Compared with the monocropping, intercropping increased the microbial utilization of carboxylic acids, amides, polymers and phenolic acids in the rhizosphere surrounding tea by 45.14%, 72.92%, 24.47%, 141.67%, and a significant difference existed in the utilization of polymers and phenolic acids. At the same time, the microbial utilization of carbohydrates, carboxylic acids, polymers and phenolic acids in the rhizosphere surrounding intercropping soybean were 1.02%, 53.19%, 81.91%, 59.13% higher than that utilized in monocropping treatment, contributing to a significant difference found in the utilization of carboxylic acids, polymers and phenolic acids.

Differences in utilizing single carbon source by soil microorganisms

It can reflect soil microbial community structure by detecting the utilization of soil microorganisms to single carbon source, which will be used to determine the microbial community functional diversity. As shown in Table 01, the utilization of soil microorganisms to α-D-Lactose, β-Methyl-D-Glucoside, D-Xylose, i-Erythritol, D-Mannitol, L-α-Glycerol Phosphate, L-Arginine, L-Phenylalanine, D-Galacturonic acid, D-Glucosaminic acid, α-Ketobutyric acid, D-Malic acid, Tween 40, Cyclodextrin, Putrescine, 2-Hydroxy Benzoic acid and 4-Hydroxy Benzoic acid in intercropping tea were higher than that utilized in monocropping treatment.
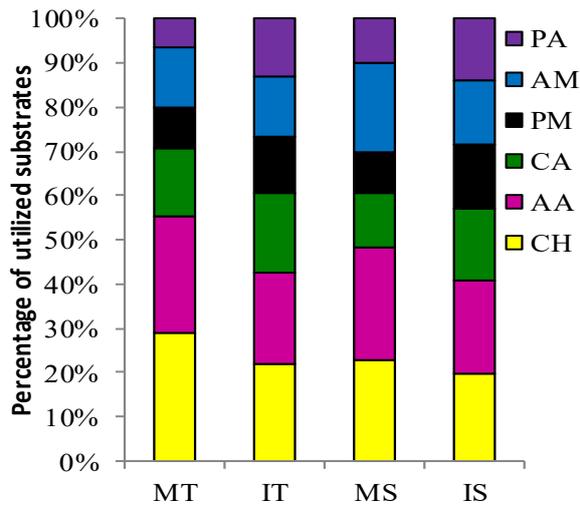
Figure 02: **Percentage of utilized substrates by soil microorganisms**
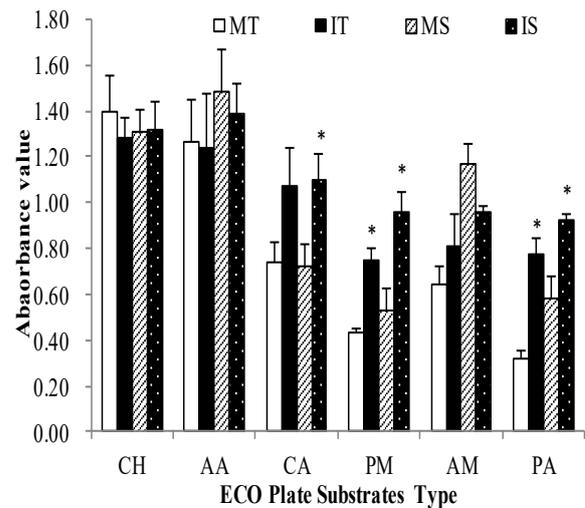

Figure 03: **Utilization intensity of soil to six types of carbon source**

Note: CH: carbohydrates; AA: amino acids; CA: carboxylic acids; PM: polymers; AM: amines/amides; PA: phenolic acids.

The microbial utilization of β-Methyl-D-Glucoside, D-Xylose, i-Erythritol, D-Galactonic Acid γ-Lactone, L-Phenylalanine, L-Serine, Pyruvic Acid Methyl Ester, D-Glucosaminic acid, D-Galacturonic acid, γ-Hydroxybutyric acid, Itaconic acid, D-Malic acid, Tween 40, Cyclodextrin, Glycogen, Putrescine and 4-Hydroxy Benzoic acid in intercropping soybean were also stronger than that utilized in monocropping treatment. Furthermore, the microbial utilization of carbohydrates and carboxylic acids were more sensitive.

Table 01: Increased Carbon source type utilized by tea and soybean under intercropping

Crops	Carbon sources
Tea	CH: α-D-Lactose, β-Methyl-D-Glucoside, D-Xylose, i-Erythritol, D-Mannitol, L-α-Glycerol Phosphate
	AA: L-Arginine, L-Phenylalanine
	CA: D-Galacturonic acid, D-Glucosaminic acid, α-Ketobutyric acid, D-Malic acid
	PM: Tween 40, Cyclodextrin
	AM: Putrescine
	PA: 2-Hydroxy Benzoic acid, 4-Hydroxy Benzoic acid
Soybean	CH: β-Methyl-D-Glucoside, D-Xylose, i-Erythritol, D-Galactonic Acid γ-Lactone
	AA: L-Phenylalanine, L-Serine
	CA: Pyruvic Acid Methyl Ester, D-Glucosaminic acid, D-Galacturonic acid, γ-Hydroxybutyric acid, Itaconic acid, D-Malic acid
	PM: Tween 40, Cyclodextrin, Glycogen
	AM: Putrescine
	PA: 4-Hydroxy Benzoic acid

Principal component analysis and cluster analysis of soil microbial diversity

Principal component analysis

To provide with a simpler interpretation of the utilization patterns for the 31 carbon resources of all treatments, we analyzed the utilization data with a partial least squares-discriminate enhance analysis (PLS-EDA). As shown in Figure 04A, there was a significant separation between monocropping and intercropping treatments on PC1, where the intercropping treatment was distributed mainly on the negative direction of PC1 and monocropping treatment was distributed mainly on the positive direction of PC1. However, no obvious difference was observed on PC2, which suggested that the carbon source utilization pattern of soil microbial community was significantly affected by intercropping. At the same time, the result indicated ten types of carbon source that were utilized strongly by soil microorganisms. The ten types of carbon source were namely, Tween 40, i-Erythritol, L-α-Glycerol Phosphate, 2-Hydroxy Benzoic Acid, 4-Hydroxy Benzoic Acid, α-Ketobutyric Acid, L-Arginine, L-Threonine, Phenyl ethylamine and Putrescine.

Initial load factors reflect the correlation coefficient between principal component and carbon source utilization; the higher the load factor, the greater effect of the carbon source on the principal component. Choi *et al.,* (1999) believes that load coefficients were greater than 0.18 or less than - 0.18 on PC1 and PC2 could be considered to have a higher load. As shown in Table 02, there were 14 types of carbon source with the higher load on PC1, mainly including carbohydrates (4), amino acids (2), carboxylic acids (3), polymers (2), amines (2) and phenolic acids (1). While there were 9 types of carbon source with higher load on PC2, it mainly includes carbohydrates (3), amino acids (2), carboxylic acids (1), amines (1) and phenolic acids (2). The above analysis indicated that carbohydrates, carboxylic acids were the sensitive carbon sources that distinguish the differences between monocropping and intercropping treatments.

Cluster analysis

Cluster analysis is used to group the abstract objects into multiple categories with similarity that could be more intuitive to show the distance relationships among the objects. As shown in Figure 05, the average well color development (AWCD) of different treatments at 120 h was for cluster analysis. Result shows that intercropping and monocropping treatments is obviously divided into two categories that suggest soil microorganisms has different carbon source utilization pattern between monocropping and intercropping treatments.
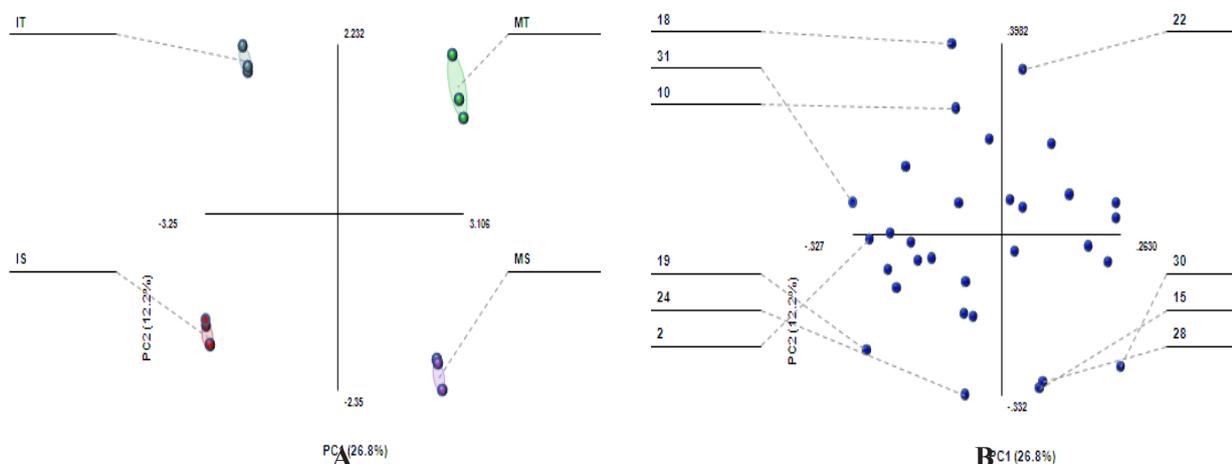

Figure 04:   Principal Component analysis of carbon utilization profiles in soil microbial community of monocropping and intercropping treatments

Table 02: **Carbon substrates most heavily loaded on first two principal components (PC) in analysis of ECO micro-plate data**

Carbon sources	Substrates	PC1	PC2
Carbohydrates	D-Cellobiose	0.25	
	D-Xylose	-0.19	
	N-Acetyl-D-Glucosamine	0.19	
	Glucose-1-Phosphate	0.25	
	α-D-Lactose		0.26
	D-Mannitol		0.19
	L-α-Glycerol Phosphate		-0.32
Amino acids	L-Asparagine	0.23	
	L-Phenylalanine	-0.25	
	L-Arginine		-0.33
	L-Threonine		-0.31
Carboxylic acids	D-Glucosaminic Acid	-0.20	
	D-Galacturonic Acid	-0.24	
	D-Malic Acid	-0.21	
	α-Ketobutyric Acid		0.34
Polymers	Tween 40	-0.29	
	Cyclodextrin	-0.23	
Amines/amides	Phenyl ethylamine	0.26	-0.27
	Putrescine	-0.33	
Phenolic acids	4-Hydroxy Benzoic Acid	-0.30	-0.24
	2-Hydroxy Benzoic Acid		0.40

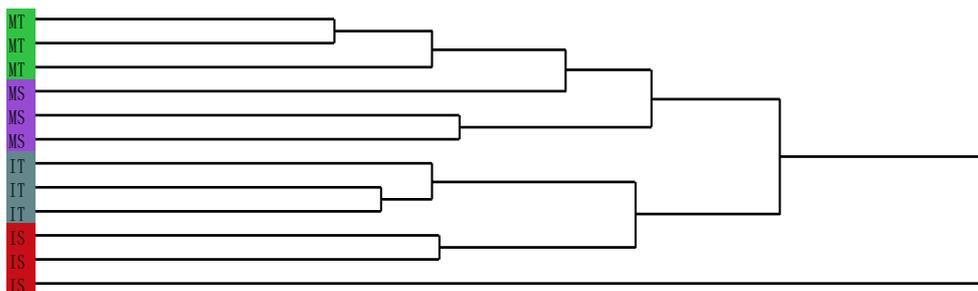The loading was > 0.18 or < −0.18.

Figure 05: Cluster analysis of carbon utilization profiles

Microbial functional diversity index

The microbial diversity assessed by Shannon index (H), Simpson index (D), Evenness index (E) and Richness index (S) are given in Table 03. These soil microbial diversity indices in intercropping treatment were greater than that found in monocropping, and increased significantly soybeans soil microbial Shannon index (H), Evenness index (E) and Richness index(S) by 7.91%, 4.85 %and 10.14% as compared with monocropping. The increased diversity indicated that the carbon source utilization patterns were more diverse and the higher richness value indicated that a larger variety of substrates were utilized in soils under intercropping.

Table 03:   Effect of intercropping on soil microbial community diversity indices

Treatment	Shannon index (H)	Simpson index (D)	Evenness index (E)	Richness index (S)
MT	3.041	0.944	0.898	29.67
IT	3.150	0.953	0.920	30.33
MS	2.971	0.945	0.908	26.33
IS	3.206*	0.956	0.952*	29.00*

DISCUSSION

Plant diversity has a significant regulation on rhizosphere microbial diversity. Crops intercropping not only can improve the shoot ecological function, but also indirectly increases soil microbial diversity of the root (Song *et al.,* 2007). Changes in AWCD values with time may be used to characterize mean microbial activity, the higher its value, the higher the metabolic activity (Konopka *et al.,* 1998), and this effect was confirmed in our study. In our study, the microbial utilization of total carbon sources in the rhizosphere around tea and soybean met the growth rule of microbial cultivation, which appeared in the adaptation phase, logarithmic phase and stable phase. In addition, we also found that intercropping treatment increased the AWCD values of soil microorganisms in comparison with those obtained in monocropping soil and these were consistent with the results of previous studies where it was found that found that tea and white clover intercropping (Xu *et al.,* 2008), wheat and faba bean intercropping (Dong *et al.,* 2013; Yang *et al.,* 2014), Soybean and Mulberry intercropping(Deng *et al.,* 2015) could improve the total soil microbial metabolic activities and functional diversity.

Soil microbial Shannon index (H), Simpson index (D), Evenness index (E) and Richness index (S) are commonly used to characterize the diversity of soil microbial, and can reveal the differences of soil microbial species and function (Tian *et al.,* 2003). Nai *et al.,* (2013) where it was found that intercropping significantly improves rhizosphere soil microbial Shannon diversity index and richness index around faba bean and wheat. When compared with monocropping. Kihara *et al.,* (2012) it also reported that microbial Shannon index (H), Simpson index (D) and Richness index (S) were higher in maize-soybean intercropping systems than that observed under monocropping. In our study, the soil microbial Shannon index (H), Simpson index (D), Evenness index (E) and Richness index (S) were higher in tea-soybean intercropping systems than that obtained under monocropping, and the increases of soil microbial diversity indices around soybean were more obvious and these were consistent with the results of previous studies. Lu and Zhang (2006) had reported that the root exudates and plant residues could provide a favorable environment as well as carbon sources and energy for the growth of soil microorganisms. So, the intercropping systems could improve the microbial community metabolic activity and functional diversity may be due to different crops root interaction which will release more abundant root exudates in intercropping system where it could provide more nutrients for growth and reproduction of soil microorganisms that promote the formation of soil microbial community structure diversity than monocropping.

In our study, the microbial utilization of carboxylic acids, amides, polymers and phenolic acids in the rhizosphere surrounding tea in intercropping treatment were greater than monocropping and the utilization of soil microorganisms to carbohydrates, carboxylic acids, polymers and phenolic acids in the rhizosphere surrounding soybean in intercropping were higher than monocropping.

Furthermore, there were 17 types of single carbon source by soil microorganisms in the rhizosphere surrounding tea and soybean in intercropping treatment were stronger than monocropping treatment respectively. Others were weaker than monocropping. It showed that utilization of these carbon sources strengthen or weaken in relation to the amounts of soil microbial population that could utilized such carbon sources to be increased or decreased and these due to the changes and accumulation in crops root exudates under intercropping may enhance the soil microbial functional diversity and may simultaneously lead to some feedback regulation.

Principal component analysis explained the differences in microbial utilization of carbon sources in different treatments. The composition of carbohydrates, amino acids and phenolic acids in root exudates was changed in rice-watermelon systems (Hao *et al.,* 2010). Carbohydrates, carboxylic acids and polymers are the main carbon sources for microbial utilization in the mulberry-soybean intercropping system that could be used as the basis for distinguishing the microbial utilization of carbon sources in different planting patterns (Li *et al.,* 2012). In our study, principal component analysis result suggested that a significant difference was found in monocropping and intercropping treatments on PC1, but no obvious difference on PC2. These indicated that tea-soybean intercropping changed the soil microbial community functional diversity. This was caused by the differences in microbial utilization of the carbon sources with higher load on the PC1, and the main carbon sources with higher load on PC1were the carbohydrates and carboxylic acids. These reflected the carbohydrates and carboxylic acids were the sensitive carbon sources to distinguish difference between monocropping and intercropping treatments that showed changes in soil microbial functional diversity in tea-soybean intercropping system. These were caused by the differences in microbial

utilization of carbohydrates and carboxylic acids that suggested that the tea and soybean intercropping may change the composition of carbohydrates and carboxylic acids.

Cluster analysis results also indicated that the soil microorganisms in monocropping and intercropping treatments were divided into two categories, and a significant difference was found in them that suggested soil microorganisms had a different carbon source utilization pattern.

CONCLUSIONS

Tea and soybean intercropping is an important practice of multiple cropping and stereo cultivation for improving tea quality and yield in China. The effects of such an intercropping system on the soil microbial properties remain unclear. In our study, the soil microbial metabolic activities (AWCD) and diversity indices in the rhizosphere surrounding tea and soybean in intercropping were increased compared with those found under monocropping. Principal component analysis and Cluster analysis results suggested that intercropping significantly changed soil microbial community metabolic activities and functional diversity depending mainly on carbohydrates and carboxylic acids. However, the microbial utilization of 6 types of carbon source had some differences. Therefore, future researches should be focused on efforts to gain in-depth knowledge about the tea-soybean intercropping system underlying the changes in soil microbial properties and root exudates, particularly the interrelation between diversity properties and tea quality.

ACKNOWLEDGEMENTS

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