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Effects of Wheat-Faba Bean Intercropping on Soil Microbial Community Structure in the Rhizosphere

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Abstract

Phospholipid fatty acids (PLFA) analysis and real-time quantitative PCR were used to investigate the effect of wheat-faba bean intercropping on soil microbial community in the rhizosphere and ammonia monooxygenase (*amoA*) gene abundances of ammonia-oxidizing archaea (AOA) and bacteria (AOB) at harvest stage via field trials in the red soil. We found that the bacteria and fungi of faba bean and actinomycetes of wheat in the rhizosphere showed significant ($p < 0.05$) difference between intercrops and monocrops at harvest. In total, 37 PLFA were detected at harvest stage in the rhizosphere, including 31 bacterial PLFA, 3 fungal PLFA, and 3 actinomycete PLFA. Compared with the rhizosphere of monocropped faba bean, a lower AOB abundance was found in the intercropping at harvest stage, whereas no significant difference in the AOB abundance was found in the rhizosphere of monocropped and intercropped wheat. There was no significant difference in the AOA abundance between monocrops and intercrops rhizosphere, but a higher AOA abundance in the intercropping systems was found. After intercropping, the abundance of AOB in rhizosphere was significantly higher than that of AOA. Our findings suggest that wheat-faba bean intercropping may change the micro-environment and microbial community structure in the rhizosphere.

Keywords

Intercropping, Phospholipid Fatty Acids, AOA and AOB, Quantitative PCR

1. Introduction

Intercropping system is a conventional and widespread practice in major Chi-

nese croplands. Intercropping, which grows at least two crop species on the same pieces of land at the same time, with >28 million hectares annually sown in China [1], is also common in other parts of the world, such as in India, Southeast Asia, Latin America, and Africa [2]. It plays an irreplaceable role in agricultural production in Yunnan Province by benefitting from the rich biological resources. Recent research shows that intercropping, compared with the monoculture cropping, could maximize crop growth and productivity [3], and decrease the accumulation of nitrate in soil profiles [4]. Intercropping cultivation could utilize resources more efficiently [5] and increase the microbial diversity in the soils [6]. Intercropping could also balance the nutrients in the soils [7], lower the damage caused by pests and diseases [8] and improve the quantity [9] and the quality [10] of products. Because global demand for food is projected to double by the 2050 year [11], the large increase in yields and land utilization efficiency from intercropping is likely to play an important role in meeting global food demand if intercropping is adopted in other regions, especially in tropical and subtropical habitats. Wheat and faba bean are traditional and abundant cash crops in China. Legume-grass intercrops are known to produce higher yields [12].

Soil nitrogen transformation in intercropping system, such as soil nitrogen fixation, release, absorption and loss, is a prerequisite for increased nutrient use efficiency, advanced yield and enhanced disease resistance of crops. The practice of leguminous-gramineous intercropping system has a long history since it can promote nitrogen uptake in gramineous crops through symbiotic nitrogen fixation [12] [13].

Soil microbial community structure is a sensitive index to evaluate soil quality and fertility. Soils with complex and rich microbial community structure indicate a stable soil ecosystem and advanced ecological function, showing great buffering capacity of the external environment. Previous research has shown that the Shannon index (H), Simpson index (D), evenness index, (E) and richness index (S) of rhizosphere microbial community in intercrops of maize and potato were higher than those in monocrops [14]. Yang *et al.* also suggested that wheat-faba bean intercropping could significantly improve the microbial carbon use efficiency and change the microbial community structure in the rhizosphere of faba bean [15]. Other research also indicated that wheat-faba bean intercropping could increase the population of bacteria, fungi, and actinomycetes in the rhizosphere [16]. Intercropping has been shown to affect therhizosphere microbial community indirectly by changing the physiological characteristics of the plant and the root exudates, and improve the soil environment by increasing the total amount of microbes [17]. Therefore, knowledge of structure and function of the soil microbial community is critical to understanding the intercropping effect on soil environment and revealing the mechanism of yield increase in intercropping systems.

2. Material and Methods

2.1. Field Site Description

The field experiment was conducted at the Experimental Site of Yunnan Agricultural University during October 2014 to May 2015. This site is dominated with red soil of pH 6.35. It contained organic matter of 14.4 g/kg, available K 116.4 g/kg, available P 24.2 g/kg, and alkali-hydrolyzed N 105.2 g/kg.

2.2. Experimental Design

The fertilizers applied to the intercropped and monocropped wheat were a composition of urea, single superphosphate, and potassium sulfate. The application rate for wheat was 225 kg·N/ha, 75 kg P_2O_5/ha, and 75 kg K_2O/ha. No organic fertilizer was applied. The application rates of N in the intercropped and monocropped faba bean were half of that in the wheat crops. The rates of P and K fertilizer in the faba bean were the same as that of wheat crops. The N, P, and K were applied once as a basal dressing on the faba bean. In the wheat field, 50% N fertilizer was applied as a basal dressing and the remainder was to pdressed twice, while P and K fertilizer were all applied once as a basal dressing.

2.3. Plant Growth Conditions

The experiment included three treatments, *i.e.*, monocropping of wheat, monocropping of faba bean, and wheat-faba bean intercropping. Each treatment was replicated four times. A total of 12 plots (each sized 4 m × 2 m) were arranged randomly. Wheat was sown by drilling with a row spacing of 0.2 m. Faba bean was sown by dibbling method with an inter-row spacing of 0.3 m and intra-row spacing of 0.2 m. We applied row-intercropping by planting six rows of wheat with 2 rows of faba bean. There were 14 rows in each intercropping plot, 19 rows in each plot of monocropped wheat, and 11 rows in each plot of monocropped faba bean.

3. Methods

3.1. Soil Samples

Two to three soil samples were randomly collected in the monocropping treatment. In the intercropping treatment, soil samples were collected from rows where wheat and faba bean met. At harvest, the rhizosphere soil was collected by shaking soils apart from the root and then was well mixed. The soil samples required for the PLFA analysis were stored at −20˚C, and soil samples of AOA and AOB test were stored at −80˚C.

3.2. Biomass and Yield of Wheat-Faba Bean at Harvest Stage

The whole plant was oven-dried at 150˚C for half-hour, then was oven-dried at 60˚C - 70˚C until constant weight, which referred to the biomass. The seeds of wheat or faba bean were collected from a 1 m^2 plot, air-dried to constant weight

to represent the yield. Land equivalent ratio (LER) was used as an index of yield advantage which was calculated using the equation:

$$\mathrm{LER} = \left(Y_{iw}/Y_{sw}\right) + \left(Y_{is}/Y_{ss}\right) \quad (1)$$

where Y_{iw} and Y_{is} represent the yield or biomass of wheat and faba bean on the total area of intercropping, respectively. Y_{sw} and Y_{ss} represent the yield or biomass of mono-wheat/faba bean on the whole area, respectively. LER value > 1 indicates an advantage from intercropping, and LER < 1 indicates a disadvantage from intercropping.

3.3. Assessment of Soil Microbial Community Structure

The soil microbial community was detected by the Phospholipid fatty acids (PLFA) analysis [18] [19]. Two grams of fresh soil samples were collected and the modified Bligh & Dyer method were applied for lipid extraction and PLFA analysis [20]. The soil samples were extracted with citric acid buffer (0.1 mol/L, pH 4.0), chloroform, and methanol in a volume ratio of 0.8:1:1.2. The extraction was acquired by silicic acid bonded solid-phase-extraction column (SPE-SI), eluted with chloroform, acetone and anhydrous methanol in sequence. The phospholipid fraction was dried with pure nitrogen gas and then hydrolyzed and saponified (methylated) with alkaline methanol to get phospholipid fatty acid methyl ester (FAME). The PLFA was determined according to the manufacturer's instructions using the MIDI method, and C19 (methyl nonadecanoate) was used as an internal standard.

3.4. Extraction of Soil DNA and Quantification of AOA and AOB by the Real-Time PCR

DNA was extracted from about 0.5 grams of fresh soil samples using the soil-specific DNA extraction kit, and the quality and quantity of DNA were tested using a NanoDrop spectrophotometer. The product of fluorescence quantitative PCR (qPCR) was determined by a fluorescence quantitative assay system. The SYBR Premix Ex Taq™ Perfect Real Time Kit (Dalian Bao Bioengineering Co.) was performed on a CFX96 Real-Time PCR System analyzer. The qPCR assays were carried out in a 20 μL reaction containing 2 μL DNA template, 10 μL SYBR Premix Ex Taq Perfect Real Time, 0.3 μL front primer, 0.3 μL rear primer, and 7.4 μL sterilized double distilled water. In the control group, sterile double distilled water was used as DNA template. The primers for ammonia-oxidizing bacteria and ammonia-oxidized archaea were ArchamoAF/Arch-amoAR [21] and Arch-amoAF/Arch-amoAR [22], respectively. The primers and PCR conditions used in nitrifying bacteria are on **Table 1**.

4. Results

4.1. Effects of Wheat-Faba Bean Intercropping on Crop Yield and Biomass

Compared with monocropped wheat, the yield and biomass of intercropped

Table 1. Primers and PCR conditions used in nitrifying bacteria.

gene	Primer sequence (5'-3')	Length of amplicon	Thermal profile for PCR
amoA AOB *amoA* gene	amoA-lF: GGG GTT TCT ACT GGT GGT amoA-2R: CCC CTC KGS AAA GCC TTC TTC	491 bp	95˚C, 3.0 min; 35× (95˚C, 0 s; 55˚C, 20 s; 72˚C, 20 s, 83˚C, 20 s with plate read); Melt curve 65.0˚C to 95.0˚C, increment 0.5˚C 0:05+ plate read
amoA AOA *amoA* gene	Arch-amoAF; TAATGG TCTGGC TTA GAC G Arch-amoAR: CGG CCA TCC ATC TGT ATG T	635 bp	95˚C, 3.0 min; 39× (95˚C, 10 s; 55˚C, 20 s; 72˚C, 20 s with plate read); Melt curve 65.0˚C to 95.0˚C, increment 0.5˚C, 0:05+ plate read

Note: M = A/C, R = A/G, W = A/T, S = G/C, Y = C/T, K = G/T, V = A/G/C, H = A/C/T, D = A/G/T, B = G/C/T, N = A/G/C/T.

wheat significantly increased by 12.6% and 15.8%, respectively ($p < 0.05$) (Table 2). But no significant effect of intercropping on the yield and biomass was found for faba bean. The LER values of grain yield and biomass in the intercropping system were all greater than one, indicating an obvious intercropping advantage.

4.2. Changes in PLFA Profiles in the Rhizosphere after Wheat-Faba Bean Intercropping

A total of 37 PLFA were identified in the rhizosphere at harvest after wheat-faba bean intercropping, including 31 bacterial PLFA, 3 fungal PLFA, and 3 actinomyce PLFA (Table 3). The results showed that the bacterial PLFA and fungal PLFA in the rhizosphere of intercropped faba bean and actinomyces of intercropped wheat were significantly ($p < 0.05$) different from those of monocrops at harvest stage.

4.3. The Principal Component Analysis of the Soil Microbial Composition PLFA

Principal component analysis (PCA) can reduce dimension mathematically to target the dominant variables through linear transformation from multiple variables. The 37 PLFA identified in the rhizosphere of wheat and faba bean under different planting patterns at harvest were analyzed to reveal the changes of microbial community and the dominant responding types of fatty acids. The first m principal components with the corresponding eigenvalues greater than one were selected as the principal component. According to the Kaiser standard, PC1 and PC2 with eigenvalues greater than one can explain most of the information of the variables.

The results of PCA are shown in Figure 1(a). The first principal component (PC1) and the second principal component (PC2) accounted for 49.3% and 16.8% variation of all variables, respectively. The PC2 showed significant difference between monocropped and intercropped faba bean, but no differences were

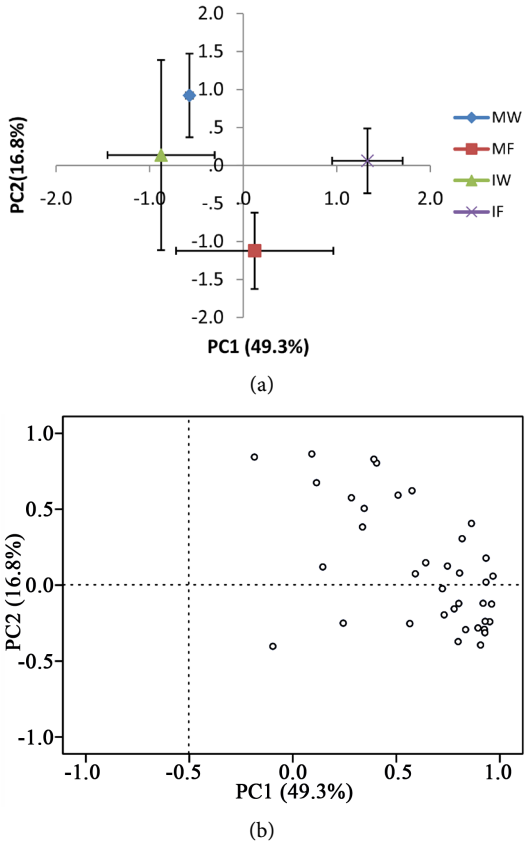

Figure 1. Principal component analysis (PCA) of the PLFA pattern from 37 PLFAs.

Table 2. The biomass and yield of wheat and faba bean at harvest (g/m^2).

Soil sample types	Wheat		Faba bean		LER
	M	I	M	I	
yield	287.46 ± 9.09b	323.68 ± 29.66a	266.18 ± 3.42a	272.60 ± 10.84a	1.09
biomass	351.75 ± 40.22b	407.33 ± 14.57a	284.15 ± 40.22a	294.20 ± 18.72a	1.12

Note: M and I indicate monoculture and intercropping; values with different small letters are significantly different between intercropping and monoculture at a significance level of 5%. The explanations of all abbreviations apply to other figures and tables.

Table 3. Types and concentrations (nmoL·g^{-1} DW) of PLFAs in the rhizosphere after wheat-faba bean intercropping.

Soil sample types	Total PLFA	Bacterial PLFA	Fungal PLFA	Actinomyce PLFA	Other phospholipids PLFA
Monocropped wheat	122.32 ± 8.12a	32.74 ± 2.90ab	9.89 ± 1.35a	1.46 ± 0.15b	74.6 ± 0.15b
Intercropped wheat	113.87 ± 22.46a	33.73 ± 3.61a	10.36 ± 1.10a	3.23 ± 0.08a	73.72 ± 0.08bc
Monocropped faba bean	95.15 ± 16.90a	13.33 ± 3.74c	6.00 ± 1.30b	2.88 ± 0.20ab	68.58 ± 0.20c
Intercropped faba bean	125.03 ± 9.64a	26.36 ± 3.38b	8.28 ± 1.87a	3.31 ± 0.41a	87.42 ± 0.41a

found between monocropped and intercropped wheat. Our finding revealed that the types of PLFA has no differences between monocropped and intercropped wheat, however, there were differences in microbial community between monocropped and intercropped faba bean. The distribution of most PLFA along the PC1 was shown in Figure 1(b), with the cumulative contribution rate over 80%. The sum of the following PLFA was used as a measure of Gram-positive bacteria (G+): 14:0 iso, 15:1 iso G, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, 18:0 10-methyl, TBSA. The sum of the following PLFA was used as a measure of Gram-negative bacteria (G−): 12:00, 12:0 iso 3OH, 16:1 w5c, 17:0 cyclo, 17:00, 18:1 w7c 11-methyl, 17:0 iso 3OH. The sum of the following PLFA was used as a measure of aerobic bacteria: 15:1 iso G, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, and 17:00. The PLFA 17:0 cyclo was used as a measure of anaerobic bacteria and 18:1 w9c was used as a measure of the fungi. The PLFA 18:0 10-methyl and TBSA were used as a measure of actinomycete.

4.4. The Ratio of Fungal: Bacterial PLFA in Soil after Intercropping

The ratio of fungal:bacterial PLFA in rhizosphere soil of mono-faba bean was higher than that in intercrops; there were no significant intercropping effects on the ratio of fungal: bacterial PLFA in the rhizosphere of wheat (Figure 2).

4.5. Changes of AOA and AOB Abundance in the Rhizosphere after Intercropping at Harvest

The gene copies of AOA and AOB in the rhizosphere at harvest after intercropping are shown in Figure 3. There were no significant differences in AOB in the rhizosphere of wheat between monocrops and intercrops at harvest. For the rhizosphere of faba bean, the AOA and AOB abundance of monocrops was greater than that of intercrops.

5. Discussion

5.1. PLFA Analysis in Soil after Intercropping

Land management practices, plant species and physiological status have a significant effect on soil microbial activity and community structure in the intercropping

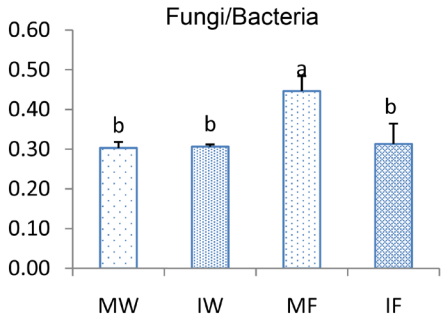

Figure 2. The ratio of fungal: bacterial PLFA in the rhizosphere after wheat-faba bean intercropping.

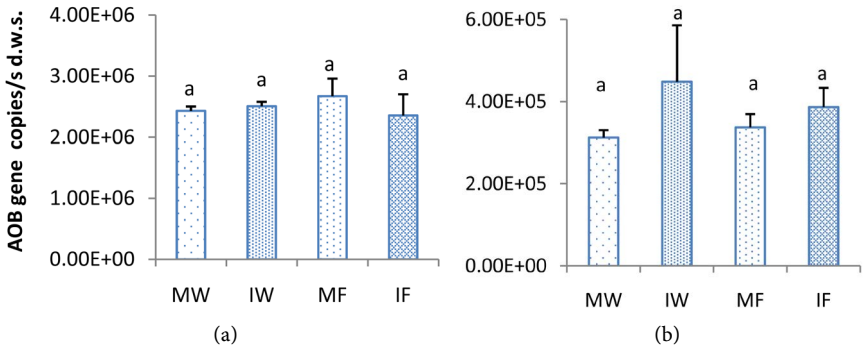

Figure 3. The gene copies of AOB (a) and AOA (b).

system [23]. The intercropping advantage was attributed to crop interactions and the changes in crop rhizosphere microbial activity [23]. In the present study, bacterial and fungal biomass in the rhizosphere of faba bean were significantly different between intercropped and monocropped faba bean ($p < 0.05$). The same pattern was found in actinomycete biomass in the rhizosphere of wheat ($p < 0.05$). Previous study indicated that a certain component of root exudates could promote the accumulation of Gram-positive bacteria and AM fungi in soil, and could also increase the total microbial biomass [24]. Root exudates contain sugars, amino acids, vitamins, and other substances, all of which could provide the nutrients and energies for the survival and reproduction of rhizosphere microorganisms [25]. Furthermore, different crops in the intercropping system could release specific root exudates, which could alter the structure of rhizosphere microbial communities to form a suitable rhizosphere environment. This could improve the overall metabolic activities of soil microbes, increase soil microbial diversification and maintain a healthy development of soil [25]. Li *et al.* used the PLFA analysis to confirm that the rhizosphere microbial community structure of maize and legume could be changed by intercropping in acidic soil [9]. Other Studies also showed that when the soil microbial community structure was richer and the microbial diversity was higher, the resistance to pathogen synthesis capacity would be also stronger [15] [26].

5.2. The Ratio of Fungal: Bacterial PLFA in Soil

The ratio of fungal: bacterial PLFA reflects the variation range of relative amounts of fungi and bacteria and their relative abundance [27] [28]. Bacteria are generally considered to be the dominant component in fertile soils. A high fungal: bacterial PLFA ratio means a stable soil ecosystem [29]. However, other studies suggested there was no correlation between fungal and bacterial biomass, land use, and land management practices [30]. In our study, the ratio of fungal: bacterial PLFA in monoculture faba bean was significantly higher than that of intercropping, but the wheat rhizosphere of monoculture and intercropping did not differ. This may be because the utilization of carbon sources in the rhizosphere microorganisms of the faba bean is greater in the intercropping system-

than that of the wheat [31]. Alternatively, the secretion of organic acids and the release of acid phosphatase in the leguminous rhizosphere in the faba bean and intercropping systemcan may have resulted in a derease in pH in the gramineous crop rhizosphere [9] [32] which is not conducive to the survival of certain microorganisms. The amount of bacteria PLFA in the rhizosphere of the wheat monoculture and intercropping system was higher than that of faba bean because the application of nitrogen fertilizer could promote the growth and reproduction of bacteria and actinomycetes [33]. The increase of the species and number of bacteria, fungi and actinomycetes contribute to the formation of microbial diversity in the rhizosphere and increase the disease resistance of plants in intercropping systems [33] [35].

5.3. Effects of Intercropping on the Abundance of AOA and AOB in the Rhizosphere

Nitrification is an important process in global nitrogen cycle and may contribute to nitrogen losses from the agroecosystem to the environment and result in water eutrophication and harmful trace gas emissions [36]. Microorganisms involved in the nitrification process are mainly ammonia-oxidizing bacteria (AOB) or archaea (AOA) and nitrite-oxidizing bacteria (NOB) [36]. In this study, the abundance of AOB was higher than that of AOA with soil pH of 6.4. Soil pH is one of the major drivers affecting the distribution of AOA and AOB populations. It was found that AOA populations were more resilient to low pH environment than AOB [37], suggesting AOB were more suitable for survival and reproduction than AOA under the soil conditions in this study. Some studies showed that AOB is the main driver of nitrification in several typical soil in China [38] [39]. The wheat-faba bean intercropping could significantly improve the abundance of AOA and AOB and also change the structure of soil microbes, but there are many reasons which can influence the structure of AOB and AOA in the rhizosphere of the intercropping system, so the reasons for the difference should not be understood as a whole.

6. Conclusion

At harvest, wheat-faba bean intercropping could increase the yield and biomass of both crops, but compared with monoculture, faba bean yield and biomass were not significantly increased with intercropping. Wheat-faba bean intercropping increased the amount of bacterial PLFA, changed the microbial community structure and the proportion of fungal: Bacterial PLFA in the rhizosphere. There was no significant difference in AOB abundance after intercropping, and the AOA gene abundance was higher than AOB, so the diversity and abundance of AOA were dominant in the soil.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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