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Effects of Maize/soybean intercropping on soil microbial community and biochemical properties

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ABSTRACT

In Field and Pot culture studies, maize (*Zea mays* L.) and soybean (*Glycine max* L.) were intercropped in 3-belowground roots interactions patterns (NS : no roots separation, HS : half roots separation with nylon net and Control: complete roots separation with plastic film). The effects of these roots separation treatments were studied on crop yields, rhizosphere microbial community composition and the relationship between soil microbial community and soil chemical properties. In 2- years field experiment, land equivalent ratio followed the order HS > NS > Control > 1. In belowground interactions, soil soluble substances interactions increased the intercrops yields (10.24 % and 10.63 % in 2018 and 2019, respectively). While in aboveground interactions the shoots interactions increased the intercrops yield (6.12 % and 2.04 % in 2018 and 2019, respectively), while, root interactions decreased the yield.

In pot culture, the soil available N, available P, electrical conductivity and soil enzymes (urease, invertase and acid phosphatase) were significantly higher in NS and HS than Control. Both phospholipid fatty acids (PLFA) based Pearson correlation and redundancy analysis showed that in treatments including soil soluble substances interactions and excluding soil soluble substances interactions, there was biggest difference in microbial community. The soil soluble substances interactions enhanced the microbial biomass and Gram-negative bacteria and reduced the physiological or nutritional stress in microbial community. The Gram-negative bacteria was significantly positively correlated with soil acid phosphatase, urease, invertase and available P. We found that soil soluble substances interactions caused major changes in microbial community in intercropping rhizosphere, it increased the microbial biomass, particularly Gram-negative bacteria, which was beneficial to the soil environment.

Key words: Above ground, below ground, biochemical properties, field study, *Glycine max*, interactions, intercropping, LER, Land equivalent ratio, maize, phospholipid fatty acid, PLFA, pot culture, root separation, soil chemical properties, soil nutrient, soil enzymes, soil microbial community, soybean, *Zea mays.*

INTRODUCTION

Intercropping is advantageous due to more efficient use of growth resources, greater ecosystem stability and higher yields (29). The aboveground and belowground interactions that give yield advantages in intercropping systems are extensively studied (1). Exploring the mechanisms of these interactions may increase the yields in intercropping systems. In aboveground intercropping interactions, the utilization efficiency of solar radiation has received most attention (11,19). In intercropping of tall and dwarf crops, the growth of dwarf crops is suppressed due to shading from tall components during the growth period (11,33). The belowground interactions between the component crops are

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more complex than overground interactions (17). The roots of component plants compete for soil space, water and nutrients resources (2,25,38). The solar radiation, soil space, water and nutrients are limited resources and the primary aims are to use these for intercropping advantages (38). The intense interspecific competition for these resources might decrease the yield in intercropping (20). The higher yields in intercropping are due to judiciously use the soil resources and sunlight (14,21,36), however, limited data is available on rhizosphere ecological processes in maize/soybean intercropping.

Soil microbiota is vital actor in feed-back processes between plant and soil environment, including nutrients cycling, plant stress signaling, stresses resistance and energy flows (26). Plants release wide variety of compounds in root exudates, which depend on host genotype, can domesticate their plant-specific soil microbial community. The root exudates can attract and accumulate the pathogenic or beneficial microorganisms (26). Plant diversity has profound impact on the soil microbial community, leading to changes in soil texture, improving soil nutrients availability and increasing soil ecological stability (7,17). It is thus obvious that the belowground interactions of intercrops not only involve the co-use of limited soil resources, but also changes the soil microbial communities and thereby, modify the soil environment.

Intercropping of maize and soybean is advantageous. Yang, *et al.* (36) and Jun, *et al.* (14) suggested that the yield advantages of maize/soybean intercropping were mainly due to aboveground interactions, which efficiently use the solar radiation, while underground interactions had no effect on yield. Conversely, the belowground interactions play major role than above ground interactions in increasing the intercrops yields (8,21), because the effects of roots interactions and changes in the soil environment are beneficial to both component crops. In this study, maize and soybean were intercropped in field experiments and pot culture. The roots of components crops were separated by different root barriers (Plastic sheet, Nylon net) to create 3-patterns of belowground interactions (NS: No roots separation, HS : Half roots separation and Control: Complete roots separation). In both field and Pot experiments, the soil microbial communities, soil biochemical properties and yields were determined. This study aimed (i). to evaluate the effects of aboveground shoot interactions, belowground root interactions and soil environment on growth and yield of intercropped maize + soybean and (ii) to determine the changes in soil microbial community to improve the soil environment.

MATERIALS AND METHODS

FIELD STUDY

The field experiment was done at Experimental Base, College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou, China (Latitude, 26°08’ N; Longitude, 119°23’ E; Altitude above sea level 52 m; Annual Rainfall 1600 mm; Maximum and Minimum temp: 9-36 °C). The experimental soil (0-20 cm) was sandy loam (soil pH value 6.2, Total N 1.32 $g \cdot kg^{-1}$, Total P 0.57 $g \cdot kg^{-1}$, Total K 8.03 $g \cdot kg^{-1}$, Alkali hydrolysable 42.91 $mg \cdot kg^{-1}$, Olsen-P 11.63 $mg \cdot kg^{-1}$, Exchangeable K 37.51 $mg \cdot kg^{-1}$).

The seeds of maize (*Zea mays* L. cv. Zhengda 12) and soybean (*Glycine max* L.cv. Pudou8008) were sown on April 6, 2018 and April 3, 2019. The intercropping system consisted of 3 rows of maize and 8 rows of soybean (row ratio 3:8). Row spacing and plant spacing of maize was 40 cm × 30 cm, soybean was 30 cm × 20 cm, and row spacing in intercropped soybean and maize was 35 cm (Fig. 1). Before crops sowing, to separate the component crops roots, Nylon Net 50 µm in half roots separation treatment and plastic film in complete roots separation treatment were buried in soil at 50 cm depth, to prevent the mixing of roots of component crops. Plot size was 18 m^2 (3 × 6 m). The treatments were replicated thrice in complete Randomised Design. Fertiliser 180 kg $N \cdot hm^{-2}$ (Urea, Half at sowing, and half was broadcasted at the jointing stage in maize), 52 kg $P_2O_5 \cdot hm^{-2}$ (Superphosphate, at sowing) and 75 kg $K_2O \cdot hm^{-2}$ (Potassium Chloride at sowing).

In intercrops, apart from the direct roots interactions, the soluble substances (microbes and root exudates in the soil) moves freely, these are called soluble substances interactions. Therefore, Maize and soybean were intercropped with no roots separation (NS), half roots separation using 50 µm nylon net (HS), complete roots separation using plastic film (Control) and mono-cropped maize and soybean (MS) (Fig. 1, Table 1).

Crop yields

At maturity, 5-plants in each row were randomly selected in all treatments, for yield attributes (100-seeds weight, number of seeds per plant) and the grain yield per plant. The land equivalent ratio (LER: Total land area of sole crops required to achieve the same yields as the intercrops) was calculated as under.

$$LER = Y_{im}/Y_m + Y_{is}/Y_s$$

Where, Y_{im} and Y_m : Yields of intercropped maize and pure maize and Y_{is} and Y_s : Yields of intercropped soybean and pure soybean, respectively. When LER > 1, intercropping system showed yield advantage (23).

To determine the contribution of different interactions in yield increase in soybean + maize intercropping, 4-treatments (NS, HS, Control and monoculture treatment) were done in field plots. The treatments, NS, HS and Control were included in aboveground interactions. In below ground interactions, soil soluble substances (microbes, root exudates) interactions and roots direct interactions occurred in NS, while, the soil soluble substances interactions occurred in HS, and No belowground interaction existed in Control (Table 1).

Yield contribution rate (*YCR*): the contribution rates of different interaction factors (aboveground interactions, roots direct interactions, soil soluble substances interactions) to intercropping yield were calculated as under:

$$AYCR_x = \frac{Y_{CSx}/R_x - Y_x}{Y_x} \times 100\%$$

$$RYCR_x = \frac{Y_{NSx}/R_x - Y_{HSx}/R_x}{Y_x} \times 100\%$$

$$SYCR_x = \frac{Y_{HSx}/R_x - Y_{CSx}/R_x}{Y_x} \times 100\%$$

$$AYCR = (AYCR_m \times R_m + AYCR_s \times R_s) \times 100\%$$

$$RYCR = (RYCR_m \times R_m + RYCR_s \times R_s) \times 100\%$$

$$SYCR = (SYCR_m \times R_m + SYCR_s \times R_s) \times 100\%$$

Where, R_m and R_s : Planting area ratio of maize and soybean in intercropping respectively, x : Single crop in intercropping, i.e. m (maize) or s (soybean). Y_{CSx}, Y_{HSx}, Y_{NSx}, and Y_x : Mean yield of x crop in Control, HS, NS and monoculture treatment, respectively. *AYCR*: Contribution rates of aboveground interactions, *RYCR:* Roots direct interactions and *SYCR:* Soil soluble substances interactions to intercrops yield.

POT CULTURE

The pot experiment was done in greenhouse (Fig. 1). The soil was sieved through 1 cm mesh to remove clods and plant debris. It was mixed with fertilizer (mg·kg^{-1} soil): N 100 ($Ca(NO_3)_2 \cdot 4H_2O$), P 150 (KH_2PO_4), K 150 (KCl), Mg 50 ($MgSO_4 \cdot 7H_2O$), Cu 5 ($CuSO_4 \cdot 5H_2O$), and Zn 5 ($ZnSO_4 \cdot 7H_2O$)]. One maize and 3-soybean seeds were sown per plastic pot (35 cm dia, 40 cm depth, with 13 kg soil) on April 2, 2019. The pot experiment focussed on belowground interactions in intercropping. To maintain consistency in crops canopy, pure crops of maize and soybean were not grown asper out previous study (28).

Table 1. Nature of Interactions occurred in Field Study and Pot culture

Abbreviation	Treatment details	Nature of Interactions
Field Study		
NS	Roots not separated	(i). Roots direct interactions, (ii). Soil soluble substances interactions, (iii). Aboveground interactions.
HS	Half roots separated (Nylon net)	(i). Soil soluble substances interactions, (ii). Aboveground interactions.
Control	Complete roots separated (Plastic sheet)	(i). Aboveground interactions.
MS	Monoculture	None
Pot Culture		
NS	Roots not separated	(i). Roots direct interactions, (ii). Soil soluble substances interactions.
HS	Half roots separated (Nylon net)	(i). Soil soluble substances interactions.
Control	Complete roots separated (Plastic sheet)	Control- no belowground interactions.

Three intercropping treatments for roots separation of components crops were: NS: No roots separation, HS: Half roots separation with nylon net 50 µm and Control: complete roots separation. All treatments had the same canopy configuration (Table 1). The treatments were replicated 6-times in complete randomised design.

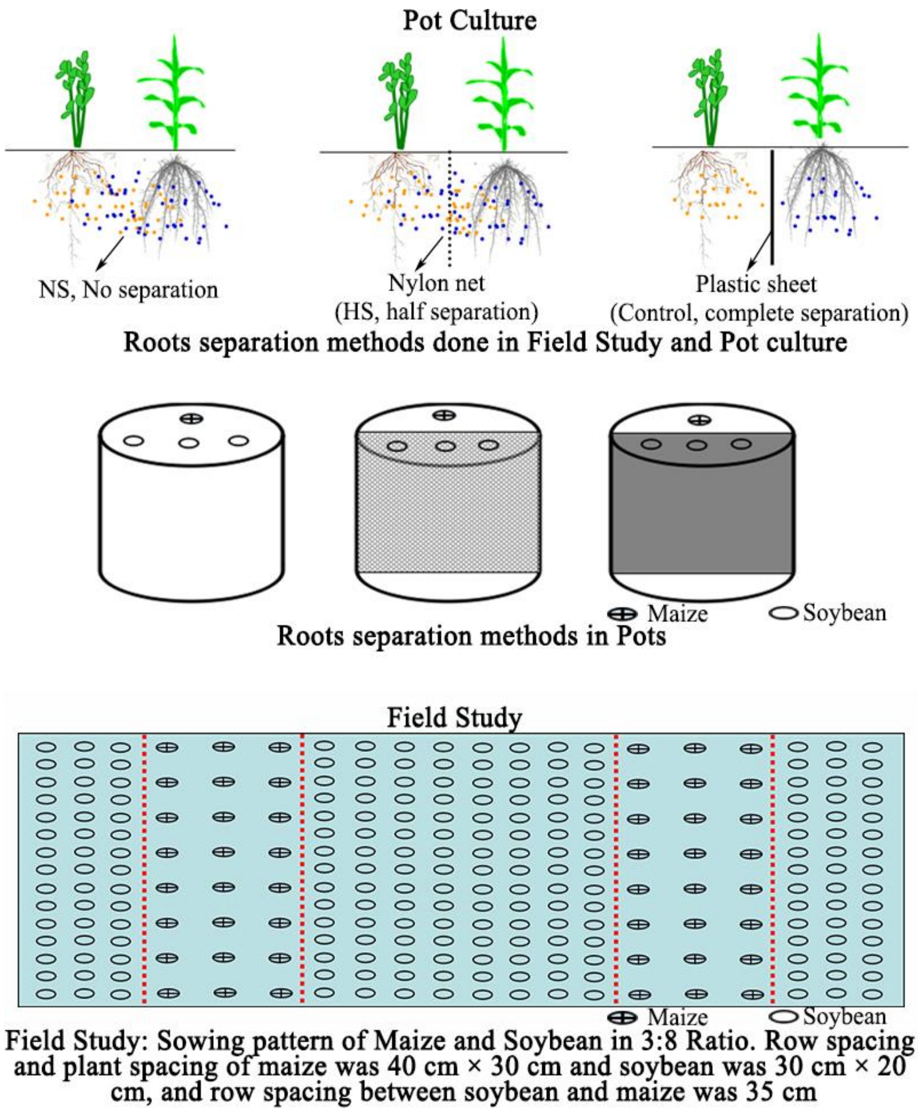

Figure 1. Root separation methods used in Maize + Soybean Intercropping systems in Field/ Pot culture and sowing pattern in filed study. Orange and Blue spots show soil soluble substances (Root exudates, soil microbes etc.) in soybean and maize soils, respectively.

Plant growth, **soil enzymes**, **soil chemical properties**

In pot culture 60 days after sowing, 4-maize and soybean plants were randomly selected. The net photosynthetic rate and relative chlorophyll contents of maize and soybean leaves were measured with Li-6400 (LI-COR, Lincoln, NE, USA) and Portable chlorophyll meter SPAD-502 (Minolta Camera Co., Osaka, Japan) respectively (28). The soil pH, electrical conductivity (EC), temperature (T) and moisture (M) at 5 cm distance (10 cm depth) around the chosen plants base were measured by portable pH meter (IQ 150, Spectrum technologies Inc., Illinois, USA) and WET sensor (WET-2, Delta-T Devices Ltd., Burwell, Cambridge, UK), respectively.

The soybean and maize plants were carefully uprooted from the soil, partitioned in shoot and root, and roots were gently shaken to remove loosely attached soil. The rhizosphere soil was collected as per previous study (28). Each soil sample was divided into 2-parts: one part stored at -80 °C for soil DNA extraction and other part was stored at 4 °C for soil enzyme and soil nutrients analysis. The plants were dried at 120 °C for 30 min and at 60 °C till constant weight, and then biomass was recorded.

Soil nutrients and enzymatic activities

Available N (AN) was determined by alkali-hydrolyzed diffusing method, available P (AP) by phosphomolybdate blue colorimetry and available K (AK) by flame atomic absorption spectrometry (12,30). Five soil enzymes viz., urease (UE), invertase (IE), catalase (CAT), acid phosphatase (ACP), and peroxidase (POD) were measured as per Guan *et al.* (9).

Phospholipid fatty acid (PLFA) analysis

The extraction and quantification of phospholipid fatty acids (PLFA) was done as per Wu *et al.* (32). Peak area was used to measure the individual fatty acid content in sample based on peak areas of internal standard 19:0 (nonadecanoic methyl ester) of known concentration. In total, 22 group specific PLFA were isolated and identified. The various fatty acids represent the different microorganisms and rule was used for fatty acid nomenclature as described previously (5,13,31,32). The bacteria (B) were represented by 12:0, 15:0, 16:0, 18:0 18:1w11c and 10Me16:0.

(i). Gram-positive bacteria (GP) by i13:0, i14:0, i15:0, i16:0, a14:0 and a16:0.
(ii). Gram-negative bacteria (GN) by 16:1w7t, 16:1w9c and cy:17:0.
(iii). Actinomycetes (A) by 10Me17:0 and 10me18:0.
(iv). Fungi (F) by 18:1w9c, 18:2w6, 9 and 18:3w.
(v). 20:4w and 24:0 were regarded as protozoan and plants, respectively.

The ratio of normal saturated to monounsaturated PLFA (NS/MS), and the ratios of total cyclopropyl PLFA to their monoenoic precursors [Cy/pre, (cy17:0 + cy19:0)/ (16:1w7 + 18:1w7)] are indicators of physiological or nutritional stress in microbial communities (22,24). The higher value of NS/MS and Cy/pre means microbes are under more stress.

Statistical analysis

Statistical analysis was carried out by DPS software version 7.05 software and analysis of variance (ANOVA) by LSD (least significant difference) test ($P < 0.05$) was used to determine significance of difference. The Pearson correlation was analyzed on the Majorbio I-Sanger Cloud Platform (www.i-sanger.com). Redundancy analysis (RDA) was carried out by Canoco for window 4.5. Redundancy analysis, a linear canonical community ordination method, was used to visualize the relationships between the species variable values (i.e., PLFA), the environmental variable gradients and the samples. Detrended correspondence analysis (DCA), an indirect gradient analysis based on segment length, was performed to determine the modality of the PLFA data and environmental variables. The CDA analysis resulted in short segment length (0.102), indicating the data set was linear and suitable for redundancy analysis (RDA). The species variables were transformed using ln (PLFA +1). Forward selection of soil properties variables was tested for significant contributions to the variation in the PLFA data using the Monte Carlo permutation test ($P< 0.05$).

RESULTS AND DISCUSSION

FIELD STUDY

Intercrops yields

There is no consistent information on the role of belowground interactions in soybean and maize intercropping regime (21,33,36). Therefore, to understand the underlying mechanisms, we investigated the yield advantages of various interactions in maize/soybean intercropping system. The 2-years field experiments showed that yield advantages (*LER*) in intercropping treatments followed the pattern of HS >NS >Control >1 The Control >1 means aboveground interactions increased the yields of component crops in intercropping, HS and NS >Control means belowground interactions increased the yields of component crops in intercropping (Table 2), i.e. both the aboveground and belowground interactions increased the intercropping yields. In calculation of yield contribution rate (*YCR*), the soil soluble substances (microbes, root exudates) interactions in belowground interactions, played a more critical role and increased the intercrops yields (10.24 % and 10.63 % in 2018 and 2019, respectively). While in aboveground interactions the shoots interactions increased the intercrops yield (6.12 % and 2.04 % in 2018 and 2019, respectively). But the roots direct interactions inhibited the intercrops yield (-6.28 % and -5.03 % in 2018 and 2019, respectively) (Table 3). These results showed that yields could be increased by widening the row spacing and shortening the symbiosis period in strong competitive intercropping systems (20,25,36). The results suggested that soil soluble substances interactions promoted the soil environment and roots direct interactions increased the soil resources competition.

The maize yield was mainly increased by aboveground interactions (16.57 % and 8.85 %) and followed by soil soluble substances interactions (6.16 % and 7.48 %); because maize is tall and dominant crop, hence, receives more solar radiation in intercropping competition (14,37). Meanwhile, soybean yield was mainly improved by soil soluble substances interactions (12.29 % and 12.21 %), but reduced by roots direct interactions (-7.94 % and -4.75 %); thus, roots direct interactions reduced the yield of weaker crop in intercropping. The shift in soil environment after the maize harvest via soil soluble substances interactions leads to yield recovery of weaker crops (25,33). Our study confirmed the hypothesis that the effect of roots direct interactions and soil soluble substances interactions on crop growth were inconsistent in belowground part of intercropping. Based on the above analysis, the effects of soil soluble substances interactions and roots direct interactions on soil environment needed to be further studied.

Table 2. Effects of treatments on crops yields and LER in Field Study.

Treatments	Y_m (kg·hm^{-1})	Y_s (kg·hm^{-1})	*LER*
2018			
NS (Roots not separated,)	10196.91±428.80ab	1895.42±89.69a	1.11
HS (Half roots separated, Nylon net)	10758.61±388.31a	2038.44±133.74a	1.19
Control (Roots completely separated, Plastic sheet)	9760.79±540.03b	1817.17±87.79a	1.06
Monoculture maize	8315.78±412.33c	1801.14±48.53a	
2019			
NS (Roots not separated,)	9982.33±544.44a	1841.15±68.59ab	1.08
HS (Half roots separated, Nylon net)	10486.17±598.60a	1923.59±113.51a	1.13
Control (Roots completely separated, Plastic sheet)	9811.50±403.19ab	1711.07±45.98b	1.02
Monoculture maize	9013.83±496.60b	1735.51±41.05b	

NS**:** Roots not separated; HS: Half roots separated (Nylon net); Control: Complete roots separated (Plastic sheet). Y_m : Maize yield; Y_s : Soybean yield. Different letters in columns show significant differences as per LSD's test ($P < 0.05$, n=3).

Table 3. Effects of various interactions on Yield contribution rate (YCR) in Field Study

Interactions	**2018**			**2019**		
	YCR_m	YCR_s	*YCR*	YCR_m	YCR_s	*YCR*
	Belowground interactions					
Soil soluble substances interactions	6.16%	12.29%	10.24%	7.48%	12.21%	10.63%
Root direct interactions	-2.95%	-7.94%	-6.28%	-5.59%	-4.75%	-5.03%
	Aboveground interaction					
Shoots interactions	16.57%	0.89%	6.12%	8.85%	-1.37%	2.04%
Comprehensive effects	19.78%	5.24%	10.08%	10.71%	6.09%	7.64%

YCR_m, YCR_s and *YCR*: Contribution of interaction factors to yield of maize, soybean monocultures and intercropping system, respectively. No separation was provided in the test plants shoots, so that interactions take place.

POT CULTURE

Plants growth and soil chemical properties

This study was done under controlled growth conditions for the accuracy of soil analysis. In pot experiment, the shoot biomass, chlorophyll content, photosynthetic rate in HS maize were significantly higher than NS, while, there was no significant difference between Control and NS. The chlorophyll content and photosynthetic rate of soybean were significantly higher in NS and HS than Control. The shoot biomass of soybean was highest in HS. The results indicated that the crops in HS showed the best growth performance than

NS and Control (Fig. 2) and showed similar trend to field experiment.

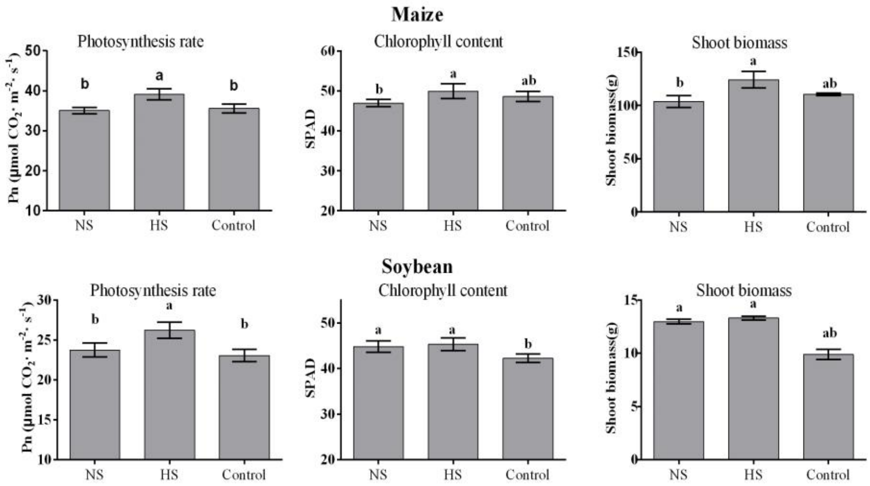

Figure 2. Effects of intercropping on Chlorophyll content, net photosynthetic rate (Pn) and shoots biomass of Maize and Soybean component crops in pot Culture. NS: Roots not separated; HS: Half roots separated (Nylon net); Control: Complete roots separated (Plastic sheet). Different letters in columns show significant differences as per LSD's test ($P < 0.05$, n=3).

Intercropping increases the soil nutrients use in comparison to monocultures (6,34). Soil enzymes are important bioactive substances in soil, which transforms and recycles the soil chemical substances and increases the soil nutrients availability (4). The soil acidification by root exudates (i.e., organic acids) in alkaline soils (17), modifies the microbial communities and higher phosphatase activity in acidic or neutral soils improves the P availability in intercropped soil (10). We found that in NS and HS, soil soluble substances interactions significantly increased the contents of available N and available P in rhizosphere soil. Similar trends were also found in soil enzymes (urease and acid phosphatase), which are involved in soil nitrogen and phosphorus cycles (Table 4). These results are consistent with studies of other intercropping systems (28,34). These results also showed that enzymatic activity and nutrients availability were sensitive to intercropping regime, the soil soluble substances interactions in maize/soybean intercropping system significantly improved the soil environment.

Effects of interspecific interactions on soil microbial community

Plant diversity determines the composition and functioning of soil biota (7). Intercropping is the specific application of plant diversity in agriculture and is beneficial to maintain the stability of soil microecology (17). In this study, the soil microbial biomass, Gram-negative bacteria, bacteria and fungi was induced in NS and HS than in Control in both maize and soybean rhizosphere soils (Fig. 3 and Fig. 4). Compared with No belowground interactions and roots direct interactions, soil soluble substances interactions

Table 4. Effects of Component crops roots separation in intercropping on Enzymes and soil chemical properties in Pot Culture

Variables	Maize			Soybean		
	NS	HS	Control	NS	HS	Control
Enzymes activities						
Urease (UE) (mg urea-N·g^{-1}·$24h^{-1}$)	0.33a	0.30ab	0.26b	0.34a	0.32a	0.28b
Invertase (IE) (mg glucose·g^{-1}·$24h^{-1}$)	2.43a	1.79b	1.10c	2.80b	3.81a	1.10c
Acid phosphatase (ACP) (mg phenol·g^{-1}·h^{-1})	0.46a	0.43a	0.37b	0.45a	0.45a	0.34b
Peroxidase (POD) (mg gallnut·g^{-1}·$2h^{-1}$)	1.33a	1.29a	1.30a	1.48b	1.54a	1.25c
Catalase (CAT) (ml KMnO4·g^{-1}·$20min^{-1}$)	0.96b	0.82c	1.03a	0.95b	0.99a	0.92b
Soil Chemical properties						
Available N (AN) (mg·kg^{-1})	80.78a	73.92b	57.77c	75.93ab	77.28ab	73.87b
Available P (AP) (mg·kg^{-1})	29.53bc	30.02bc	26.56d	31.18b	33.8a	28.79c
Available K (AK) (mg·kg^{-1})	46.39cd	44.62d	38.08e	50.06c	65.85a	59.45b
Electrical conductivity (EC) (mS·m^{-1})	103.60a	92.20b	87.80b	90.60b	96.60b	87.40a
Temperature (T) (°C)	21.22ab	21.06ab	21.00ab	21.32a	20.96b	21.06ab
Moisture (M) (%)	23.86bc	23.44bc	24.82ab	23.30bc	22.00c	26.80a
pH	6.41a	6.11a	6.13a	6.28a	6.17a	6.31a

NS**:** Roots not separated; HS: Half roots separated (Nylon net); Control: Complete roots separated (Plastic sheet). Different letters in columns show significant differences as per LSD's test ($P < 0.05$, n=3).

increased the population of total PLFA, bacteria, Gram-negative bacteria, actinomycetes and fungi (Fig. 5). In addition, the biggest difference in the microbial community was between treatments including the soil soluble substances interactions (NS and HS) and treatment excluding soil soluble substances interactions (Control) (Fig. 6). Another study also found that intercropping watermelon with rice increased the abundance of bacteria, Gram-negative bacteria and actinomycetes in rhizosphere soil, and reduced the density of pathogen *Fusarium oxysporum* (27). Recent research has shown that plants could change the composition of root exudates in response to neighbouring plants, which mediated the rhizosphere ecological processes and reshaped the soil microbial community (15). Plants diversity increases the amount and diversity of root exudates in soil, which was positively associated with higher soil microbial biomass and diversity (7,37). Our findings strongly suggested that the interactions of root exudates were the main factor in soil soluble substances interactions, driving the changes in rhizosphere microbial community in intercropping system. However, current knowledge regarding the metabolic profiling of root exudates and how the root exudates changes the soil microbial community under intercropping is limited.

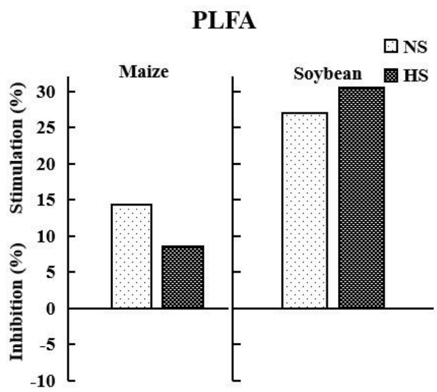

Figure 3. Effect of intercropping on PLFA (phospholipid fatty acids) in maize and soybean soil. NS: Roots not separated; HS: Half roots separated (Nylon net).

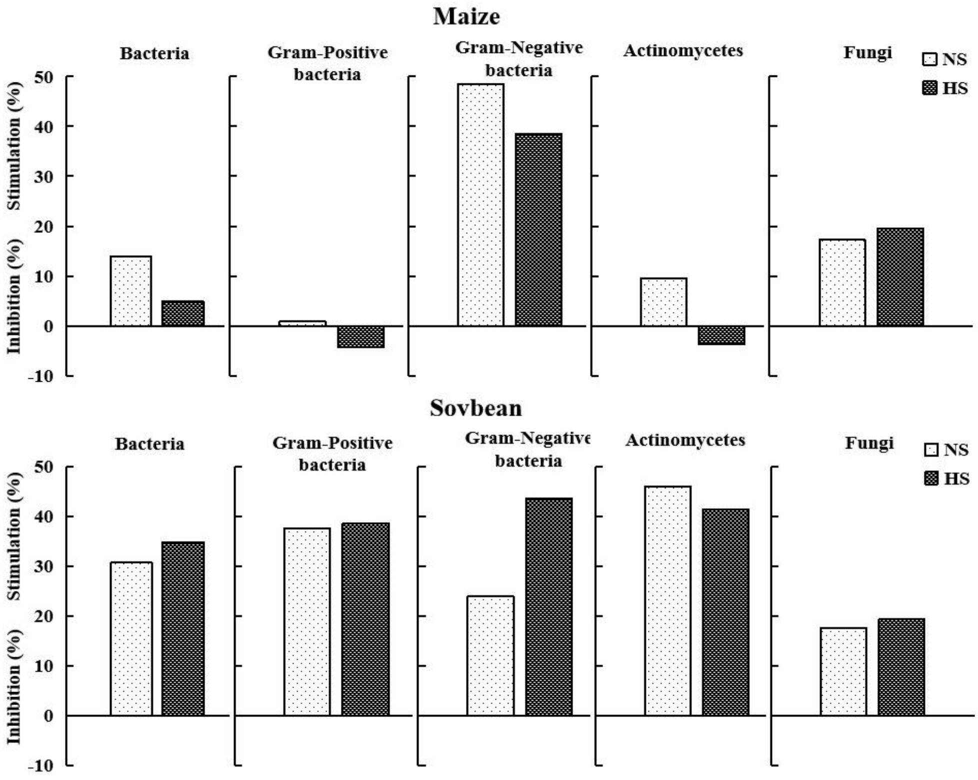

Figure 4. Effect of intercropping on microbes in maize and soybean soil. NS: Roots not separated; HS: Half roots separated (Nylon net).

Correlations between soil microbial community and soil chemical properties

Soil Microbes play dominant role in soil nutrients cycling and their availability. The higher soil microbial biomass stimulates the soil N mineralization (18). Soil enzymes (mainly derived from soil microbes) and their activities increased the soil available N, P and K (34). In this study, the urease, invertase and acid phosphatase were positively correlated with total PLFA, bacteria and Gram-negative bacteria. The available N and available P was significantly positively correlated with Gram-negative bacteria (Fig. 5). In Monte Carlo permutation test, acid phosphatase, urease, invertase, electrical conductivity, soil soluble substances interaction, No belowground interaction, and moisture were strongly and significantly ($P < 0.05$) correlated with the variation in redundancy analysis ordination of soil microbial community, which accounted for 55 %, 25 %, 30 %, 31 %, 32 %, 49 %, and 49 % of total variance in the PLFA data, respectively. Especially, the Gram-negative bacteria was increased simultaneously by soil soluble substances interactions in both maize and soybean soil (Fig. 4), and was significantly positively correlated with urease, invertase, acid phosphatase, peroxidase, electrical conductivity, available P and available N (Fig. 5). The redundancy analysis further suggested that the activities of urease, acid phosphatase and invertase were improved by soil soluble substances interactions in NS and HS, and positively correlated with Gram-negative bacteria (Fig. 6). The Gram-negative bacteria is more easily induced by low molecular weight organic compounds in root exudates (16). The Gram-negative bacteria was greatly increased in peanut/medicinal plant (*Atractylodes lancea*) intercropping system and maize-based intercropping systems, which was responsible for increased available P and had the potential to reduce the accumulation of allelochemicals (3,28,35). Our findings suggested that the drastic changes in rhizosphere microbial community in intercropping, particularly Gram-negative bacteria improved the soil environment.

NS/MS and Cy/Pro were indicators of physiological or nutritional stress in microbial communities, which decreased with restoration of ecosystem in prairie and forest soil, while microbial biomass was increased (22,24). In this study, the value of NS/MS was lowest in HS maize and soybean soil, meanwhile, Cy/pro was highest in Control soybean soil (Fig. 6). The redundancy analysis also found that soil soluble substances interactions were negatively correlated with NS/MS and Cy/pre and correlated positively with most environmental variables and species variables. Therefore, the soil microbial communities of both crops were subjected to more severe physiological or nutritional stress in roots direct interactions and No belowground interactions than soil soluble substances interactions. The observations were accounted for lower total PLFA and poor soil environment in Control, which might be related to specific root exudates released by solo crop.

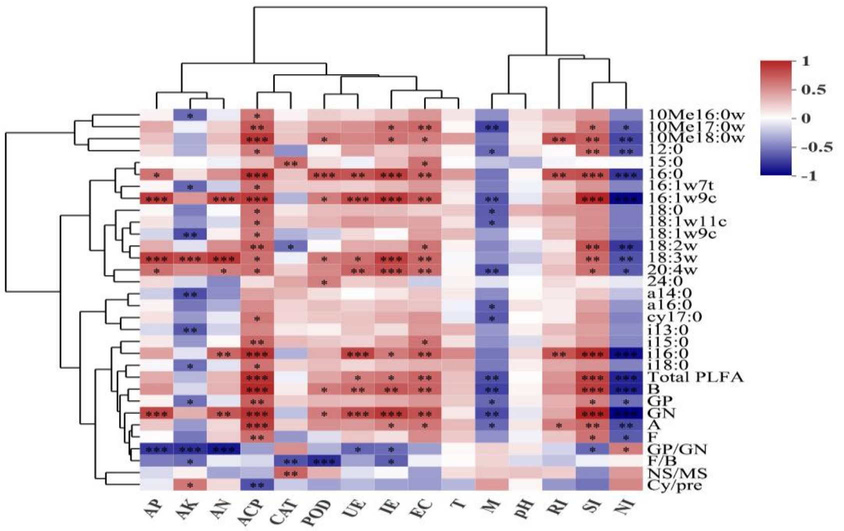

Figure 5. Pearson correlation heatmap of PLFA data *vs.* environmental variables in pot experiment. The legend on the right is the color interval of different R values.* P < 0.05, ** P < 0.01, *** P < 0.001.

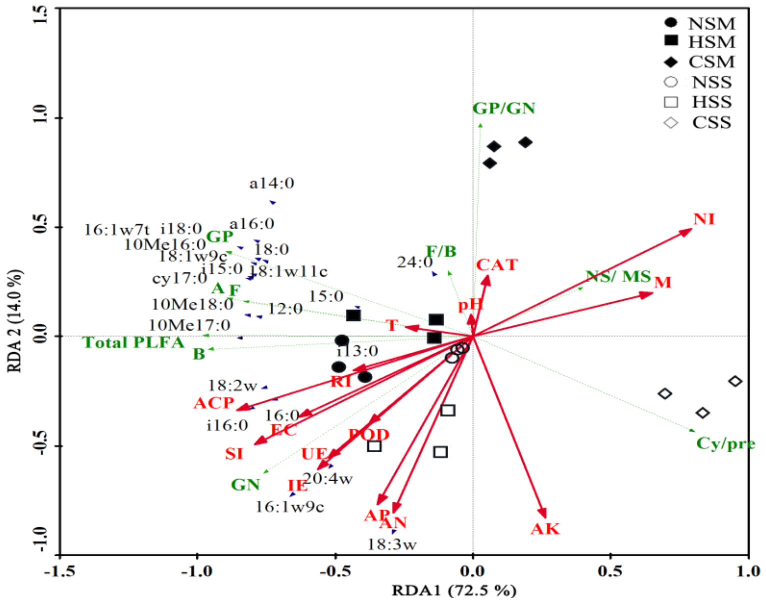

Figure 6. Effects of intercropping on Redundancy analysis (RDA) of PLFA data *vs.* environmental variables in pot culture. Blue arrowheads: PLFA data. Red solid arrows: Vectors predictor variables associated with variation in microbial community structure. Green dotted arrows: Group specific PLFA. NSM: No roots separation maize; HSM: Half roots separation maize; CSM: Complete roots separation maize; NSS: No roots separation soybean; HSS: Half roots separation soybean; CSS: Complete roots separation soybean; NI: No belowground interaction; RI: Roots direct interactions; SI: Soil soluble substances interactions.

CONCLUSIONS

Our studies found that roots direct interactions and soil soluble substances (microbes, root exudates) interactions had opposite effects on intercrops yield advantage in belowground part of maize/soybean intercropping system. The advantages of maize/soybean intercropping were mainly due to soil soluble substances interactions. The major changes in rhizosphere microbial community were induced by soil soluble substances interactions in intercropping, among which higher microbial biomass and Gram-negative bacteria population improved the soil nutrients availability and soil health. Redundancy analysis further verified that Gram-negative bacteria was positively correlated with soil acid phosphatase, urease, invertase and available P. Besides, the soil soluble substances interactions reduced the physiological or nutritional stress in soil microbial communities of both crops than roots direct interactions and no belowground interactions. The results provided insight into rhizosphere biological processes in plant-microbial-soil system in intercropping system.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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