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*Full Length Article*

# The Dynamics of Soil Microbe Metabolic Function Diversity in the Root-Zone of Maize-Soybean Intercropping

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## Abstract

Maize and soybean intercropping can increase aboveground productivity and land use efficiency. Soil microbes play important roles in plant nutrient availability and soil ecosystems. However, the dynamics of soil microbe metabolic function diversity of intercropping have been less reported. We monitored the soil microbes metabolic function, diversity and carbon sources utilization in the root-zone soil of intercropped maize and soybean throughout the growing season by using Biology techniques. Our results showed that at the seedling stage for maize and soybean, the C-substrate-utilization profiles were grouped according to crop type (i.e. intercropping maize and monocropping maize grouped together). In contrast, at the tasseling stage for maize, the profiles were grouped by cropping systems (*i.e*., intercropping maize and intercropping soybean grouped together). Furthermore, intercropping enhanced the C-substrate-utilization profiles in maize, leading to more efficient metabolism of nitrogen compounds (such as amines) and other substrates that are typically resistant to degradation (such as esters). Last, the alpha-biodiversity Shannon index (H) and Simpson (J) were significantly higher in intercropping maize (H=4.762, J=0.961) samples than in monocropping maize (H=4.685, J=0.958) at the tasseling stage for maize. 

**Keyword:** Maize-soybean intercropping; Root areas; Microbial metabolic profiling

## Introduction

Intercropping is one of the most important cropping systems in sustainable and productive agriculture (Lithourgidis *et al*., 2011). Increasing evidence indicates that intercropping of soybeans and maize can produce greater yields per unit area than growing the two crops separately *via* monocropping (Echarte *et al*., 2011; Lithourgidis *et al*., 2011; Ijoyah *et al*., 2013). Intercropping even can significantly increases the crude protein (CP) content of maize (Toniolo *et al*., 1987). At the same time, another study investigating that the yield-increasing effect was different with different soybeans and maize intercropping ratios (1:1, 2:2 and 1:2), of which the ratio of 2:2 or 1:2 produced higher stover and haulm yields in late-season maize (Undie *et al*., 2012).

Soil microbes are integral components of the soil ecosystem and play important role in plant nutrient availability (Paul and Clark, 1989). The abundance, composition, and activity of soil microbes largely determine the sustainability and productivity of agricultural land (Heijden *et al*., 2008). Agricultural practices, such as residue incorporation, cropping sequence, and intercropping, affect the soil microbial community composition (Anderson and Gray, 1990; Zhou *et al*., 2011). Loss of soil biodiversity and simplification of soil community composition can impair multiple ecosystem functions, including nutrient retention and cycling (Wagg *et al*., 2014). Microorganisms play a very important role in the cycling of almost all of the major plant nutrients. Any change in the activity or diversity of soil microbes may cause changes in the soil quality, including changes in terrestrial ecosystems' nutrient availability, plant growth, and carbon budget (Francis *et al*., 1982; Das and Chakrabarti, 2013). Furthermore, soil microbial communities may decompose carbon substrates that are specific to their soil environment (Orwin *et al*., 2006). Thus, the soil microbial community can use different carbon sources to respond to changes in the soil environment.

The Biolog EcoPlate™ (BIOLOG Inc., Hayward CA., USA), which was originally used by (Garland and Mills, 1991), was created specifically for community analysis and ecological studies of microbes. This approach can be used for community-level physiological profiling (CLPP) and provides a useful measurement of a microbial community's physiological and metabolic potential, based on the community's ability to metabolize various

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carbon sources. It is suitable for monitoring changes in soil microbes. Soil functional diversity was commonly used as an indicator for soil quality, can help us to better understand the relationship between microbial diversity and soil function (Xiao *et al*., 2016).

The primary objectives of this study were as follows: (1) to identify and compare the heterotrophic activity and functional diversity of soil microbes in root areas of intercropping maize (IM), monocropping maize (MM), intercropping soybean (IS) and monocropping soybean (MS); (2) to understand how intercropping affects microbial activity and physiological profiles.

## Materials and Methods

### Experimental Plot Planting

This study was carried out in the field plots of Yunnan Agriculture University located in Kunming City, Yunnan province, southwest China. The experimental field, which is composed of lateritic red soil, was divided into nine plots, each with an area of 16.0 $m^2$. To avoid edge effects, an alley was left between the plot and plot, at the same time a guard row was left on each side of plot. The experiment had three treatment plots: soybean only (Huayan NO.1), maize only (*Zea mays* L. cv. Yunrui NO.6) and an intercrop of soybean and maize. Each treatment was replicated three times in a randomized complete block design. Monocropped soybean was planted with 35 cm x 30 cm spacing, monocropped maize was planted with 30 cm x 40 cm spacing, and intercropped maize and soybean consisted of two rows of maize alternated with two rows of soybean (2:2 intercrop), with 30 cm row spacing between maize and soybean, 35 cm x 30 cm spacing between soybean and soybean, 30 cm x 40 cm spacing between maize and maize. Both maize and soybean were directly sown in May. Farmyard manure as a base fertilizer was applied before sowing.

### Soil Sampling and Soil Chemical and Physical Property Assays

Soil samples were collected once a month for a total of four months throughout the growing season, namely, June-the maize seedling stage (soybean seedling stage), July-the maize jointing stage (soybean seedling stage), August-the maize tasseling stage (soybean flowering and podding stage) and September-the maize ripening stages (soybean seed expanding stage). Five plants (maize or soybean) were randomly selected from every plot: 2 cm of the soil surface was removed, the soil surrounding the plant root was collected and generally within 5 cm of the root surface (20-40 cm depth) and mixed into four uniform soil samples representing different treatments. The treatments were as follows: soil in root areas of intercropping maize (IM), soil in root areas of intercropping soybean (IS), soil in root areas of monocropping maize (MM), and soil in root areas of monocropping soybean (MS). Every treatment had three biological replicates. Before the experiment began, we examined the physical and chemical properties of the soil from each plot. We measured the amount of organic matter (potassium bichromate-dilution heat colorimetric), total nitrogen (Kjeldahl), total phosphorus (NaOH-molten molybdenum), total potassium (NaOH-molten atomic absorption spectrophotometry), nitrate (Alkali N-proliferation method), available P (0.50 mol/L sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric method), and available K (ammonium acetate and atomic absorption spectrophotometry) (Rukun, 2000). We found no initial differences in these parameters among the experimental plots. At maize tasseling stage (soybean flowering and podding stage), ammonium nitrogen (nessler's reagents spectrophotometer), nitrate nitrogen (phenol disulfonic acid spectrophotometry), nitrite nitrogen (N-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometry) and soluble organic nitrogen content (the difference of total nitrogen and inorganic nitrogen) were determined by Yunnan Tongcuan Agricultural Analysis Testing Technology C.

### Preparation of Ecoplates

Soil moisture content was measured by drying the soil at 105°C for 24 h. Next, the fresh-soil equivalent of 5 g (dry weight) from each plot was mixed with 45 mL of sterile 0.85% saline and shaken at 250 rpm for 30 min (25°C) to release bacteria from the soil particles. One milliliter of this soil suspension was used for serial ten-fold dilutions in sterile saline buffer. To minimize background absorbance, soil suspensions were diluted 1000-fold with 0.85% saline under aseptic conditions. Finally, 0.15 mL of the diluted bacteria suspension was used to inoculate BIOLOG EcoPlates (BIOLOG, USA). For each soil sample, three technical replicates were prepared. The plates were incubated at 25°C in the dark, and the absorbance (590 nm and 750 nm) of the 96 wells was recorded every 24 h for 144 h using an automated microplate reader (BIOLOG, USA) (Jin *et al.*, 2012).

### Kinetic Profiling

For each well in the BIOLOG EcoPlates, we analyzed the kinetic profile of the average well color development (AWCD). Based on the sigmoidal shape of the curves, a density-dependent logistic growth equation could be fitted. Statistical Package for Social Science software (SPSS 13) was used to fit a non-linear curve. Because the CLPP of different treatments offers three different parameters of color response (K, p, s), we used these three parameters for statistical analysis of the physiological profile of the microbial community. The following equation was used to calculate the AWCD (Salomo *et al*., 2009):

$$Y = AWCD = K/(1+\exp(-p\ (t-s))$$

Where K is the asymptote/carrying capacity, p is the exponential rate of AWCD change, s is the time when y=K/2.

**Community-level Physiological Profiling (CLPP) Analysis**

The color intensity, which is a measure of the metabolic activity of a community, was expressed for each soil sample as the AWCD. The AWCD was calculated for the 31 substrates of the parallel *j* measured at time *t*, when the optical density (OD) (*i, j, t*) was the corrected OD for well *i* of parallel *j* at time *t* (Salomo *et al*., 2009):

$$AWCD_{j,t} = \frac{1}{31}\sum_{i=31}^{31} OD(i, j, t)$$

The absorbance values were normalized by dividing each color score by the AWCD value of the appendant parallel as recommended by Garland (1996).

The microbial metabolic activity was expressed using the 590 nm optical-density value minus the 750 nm optical-density value (set to 0 if less than the blank). The optical-density data were corrected by subtracting the initial color development in the plate's control well.

$$AWCD\ (590\sim 750\ nm) = \sum(C\ 590 - 750)/31$$

We selected AWCD values at two time points to calculate the Richness (R) and Shannon–Weaver index values (H), and perform principal component analysis (PCA) and analysis of variance. The first time point we chose to measure was 48 h, when the substrate utilization rates of all of the samples were transitioning from the lag phase to the exponential phase. The 120 h time point was selected as the second point for validation, because carbon substrate utilization is in the log phase at that point.

Richness (R) values were calculated as the number of oxidized C substrates, and the Shannon–Weaver index values (H) (*i.e*., the richness and evenness of the response) were calculated using an OD of 0.25 as the threshold for a positive response (Garland and Mills, 1991). Microbial community diversity was assessed using the Shannon–Weaver index and calculated using the following formula:

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

Where $p_i$ is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates $\sum OD_i$. Plate readings at 12 h of incubation were used to calculate AWCD, R, and H, because this was the shortest incubation time that allowed for optimal resolution among the treatments.

To simplify direct comparisons, we used the following seven groups of carbon substrates: (1) carbohydrates with phosphate rest (CHP); (2) carbohydrates without phosphate rest (CH); (3) carbonic acids (CA); (4) polymers (PM); (5) amino acids (AA); (6) amines (AM); and (7) esters (ES) (Salomo *et al*., 2009).

**Data Analysis**

We selected absorbance values at a single time point for analysis, and the data for each plate were initially normalized to the average AWCD. The normalized absorbance for well k was calculated as recommended by Garland (1996).

$$OD_{norm} = OD\ (i,j,t)\ /AWCD\ (j,t)$$

Fisher's least significant difference (LSD) test was used to determine if there were differences between the communities using SPSS 13. Wilks' *F*-test was first used to test the null hypothesis of no difference between any of the communities, and then Hotelling's $T^2$ test was used for all pairwise comparisons if the null hypothesis is rejected by the first test.

We performed principal component analysis (PCA) to analyze normalized and transformed absorbance CLPP data for each well using SPSS 13.

## Results

**Chemical and Physical Properties of Intercropped Versus Monocropped Soil**

The function of microbial community is influenced by soil chemical and physical properties, in which N is an important element for plant growth. Therefore, the analysis of the effects of intercropping on soil physical and chemical properties can help to comprehensively understand the effects of intercropping on soil microbial metabolism. Firstly, there were no significant differences between the plots in terms of any of the nutrients we measured before planting corn (Table 1). Then the ammonium nitrogen, nitrate nitrogen, and soluble organic nitrogen content were determined three months after planting. As shown in Fig. 1, all forms of nitrogen were highest in the monocropped soybean soil (MS). The intercropping system significantly increased the nitrate nitrogen ($NO_3^-$-N) and nitrite nitrogen ($NO_2^-$-N) content surrounding the roots of intercropped maize relative to monocropped maize (MM). But intercropping did not significantly affect the contents of ammonium nitrogen ($NH_4^+$-N).

**Utilization Rate of the Carbon Sources**

By observing and analyzing the monitoring data, we found that the microbial communities AWCD for substrate metabolism had a nonlinear correlation with incubation time (Fig. 2). Additionally, the time-course of AWCD variation is similar to that of kinetic models of microbial population growth (*i.e*., an S-curve). Therefore, a modified logistic model was used to fit the data.

**Table 1:** Soil physicochemical characteristics

| Soil sample | Organic C (g kg$^{-1}$) | Total N (g kg$^{-1}$) | Total P (g kg$^{-1}$) | Total K (g kg$^{-1}$) | Nitrate N (mg kg$^{-1}$) | Available P(mg kg$^{-1}$) | Available K (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| IP | 9.61(1.98)a | 0.55(0.08)a | 0.53(0.02)a | 10.70(0.65)a | 100.16(10.40)a | 4.02(0.86)a | 211.15(63.49)a |
| MMP | 9.41(1.77)a | 0.61(0.13)a | 0.52(0.01)a | 10.30(0.12)a | 85.17(18.05)a | 4.92(1.16)ab | 199.99(38.24)a |
| MSP | 10.64(0.50)a | 0.56(0.07)a | 0.52(0.03)a | 10.48(0.63)a | 95.77(13.80)a | 6.51(0.74)b | 174.73(17.42)a |

Note: IP= Intercropping Plot, MMP=Monoculture maize plot, MSP=Monoculture soybean plot
Standard deviations are given in parentheses. Values within the same column not followed by the same letter differ significantly ($P < 0.05$)

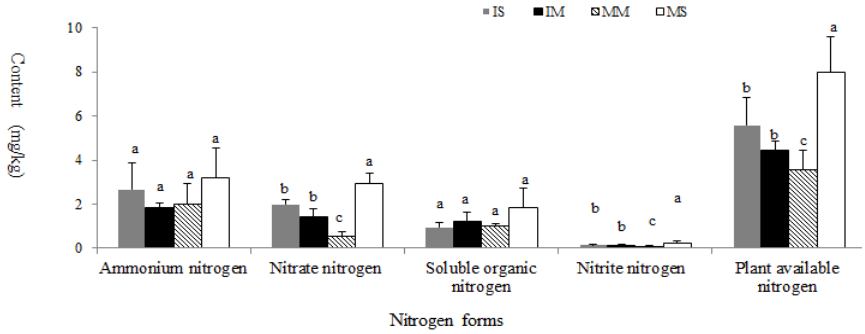


**Fig. 1:** Content of various nitrogen forms at maize tasseling stage (soybean podding stage)
IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean). Different letters indicate significant differences at $P < 0.05$

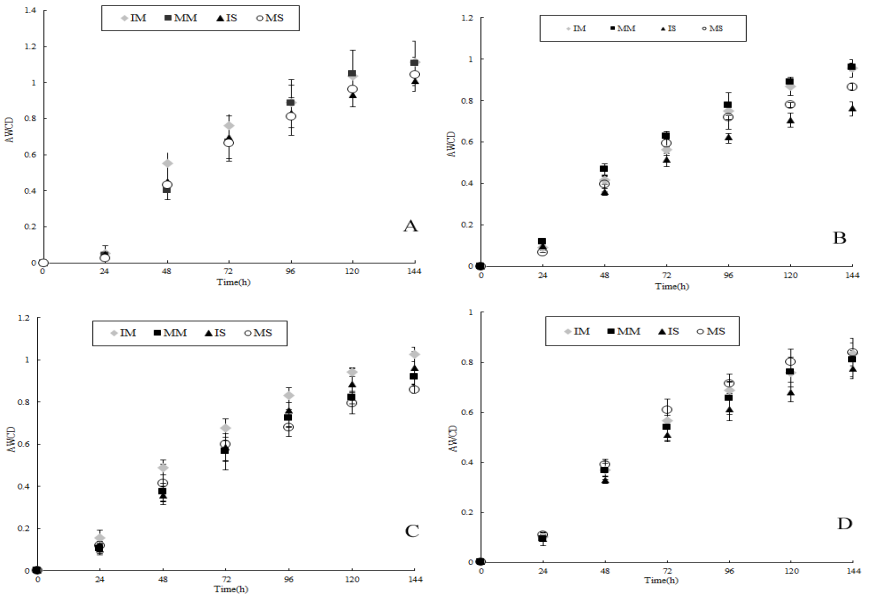


**Fig. 2:** The time course change of average well color development (AWCD) in all soil samples
Arithmetic means and their standard deviation are displayed; A: June; B: July; C: August; D: September. IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean)

Detailed values of curve integration were obtained and are reported in Table 2. The correlation coefficients obtained from the data ($r^2 \geq 0.973$, Table 2) indicated that the change in AWCD values fitted the logistic growth model well. In the logistics equation, the slope (K) represents the benefit rate of the microbial community to the carbon source. Comparing the K value across months, the K value of the IM, IS and MS first decreased from the maize seedling stage (soybean seedling stage) to the maize ripening stages (soybean seed expanding stage), followed by an increase at maize tasseling stage (soybean flowering and podding stage), and then decrease again at maize tasseling stage (soybean flowering and podding stage). Except at the seedling stage, IM soil had the higher K value than MM, therefore the higher utilization rate of the carbon sources.

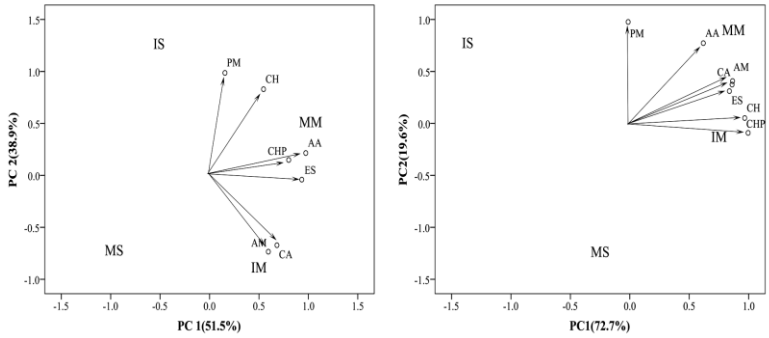


**Fig. 3:** PCA of normalised OD data at maize seedling stage (soybean seedling stage) of $AWCD_{48h}$ (left) and $AWCD_{120h}$ (right)
PC (principal component with proportion of total variability [%]); IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean); Substrates were classified in seven different carbon source categories: AA (amino acids), AM (amines), CA (Carbonic acids), CH (carbohydrates), CH.P (carbohydrates with phosphate rest), ES (ester), PM (polymers)

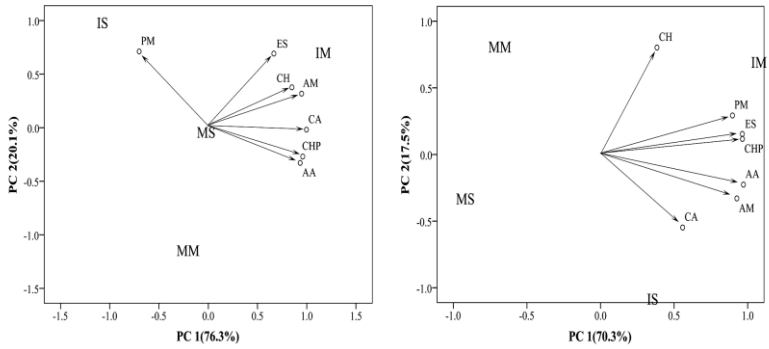


**Fig. 4:** PCA of normalized OD data at maize tasseling stage (soybean flowering and podding stage) of $AWCD_{48h}$ (left) and $AWCD_{120h}$ (right)
PC (principal component with proportion of total variability [%]); IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean); Substrates were classified in seven different carbon source categories: AA (amino acids), AM (amines), CA (Carbonic acids), CH (carbohydrates), CH.P (carbohydrates with phosphate rest), ES (ester), PM (polymers)

**Carbon Source Utilization Patterns of Soil Microbe**s

To distinguish the effects of intercropping on the catabolic diversity of soil microbes, separate PCAs for the treatments at the same sampling time and the same incubation time were performed (Fig. 3 and 4). Our results showed that at maize seedling stage (soybean seedling stage) (Fig. 3), the carbon source utilization profiles of the microbial communities at the 48 h and 120 h incubation times were similar between the IM and MM soil, while similar between the IS and MS, which showed that the metabolic function of microbial community in the root zone is mainly affected by crop specie at maize seeding stage. The $AWCD_{48h}$ PCA plot reflects the actual microbial community function. As the orientation of the arrows in Fig. 3 (left panel) illustrates, the differences between the four samples were primarily determined by the catabolism of carbohydrates with phosphate rest, amino acids and esters. The arrows representing these three substrate classes are parallel to the PC1 axis, and the differences in these three values describe 51.5% of the total variability in the data. The physiological potential of the MM microbial community was mainly determined by catabolism of amino acids and carbohydrates with phosphate restriction, while the IM community's profile was mainly determined by carbonic acid and amine catabolism (Fig. 3 and 4). The later $AWCD_{120h}$ time point reflected the potential function of the microbial community. At this time point, the differences between the four samples were primarily due to differences in the utilization of carbonic acids, amines, and esters (Fig. 3). The remaining carbon substrate classes of amino acids were mainly utilized by MM soil microbes. However, IM soil microbes mainly utilized carbohydrates and carbohydrates with phosphate rest. None of the investigated substrate classes we tested was utilized very well in the MS soil, which showed large differences in comparison with the other samples (Fig. 3).

We next performed PCA on samples gathered at maize tasseling stage (soybean flowering and podding stage). At the 120 h timepoints, the IM and IS microbial communities' carbon-source utilization profiles showed greater similarity than at maize and soybean seedling stage, which showed that the metabolic function of microbial community in the root zone is mainly affected by

**Table 2:** Kinetic parameters of the fitted logistic growth equations for four months

| Treatment | K (AWCD) | S（h） | P | $r^2$ |
|---|---|---|---|---|
| 6-IM | 1.057 | 53.324 | 0.057 | 0.954 |
| 6-MM | 1.103 | 64.142 | 0.051 | 0.984 |
| 6-IS | 0.959 | 54.920 | 0.060 | 0.973 |
| 6- MS | 1.011 | 60.634 | 0.052 | 0.976 |
| 7- IM | 0.964 | 63.261 | 0.041 | 0.976 |
| 7- MM | 0.948 | 55.253 | 0.045 | 0.974 |
| 7- IS | 0.747 | 54.100 | 0.048 | 0.981 |
| 7- MS | 0.822 | 53.393 | 0.057 | 0.975 |
| 8- IM | 1.017 | 55.126 | 0.043 | 0.983 |
| 8- MM | 0.912 | 61.079 | 0.043 | 0.986 |
| 8- IS | 0.969 | 63.725 | 0.044 | 0.992 |
| 8- MS | 0.833 | 53.108 | 0.047 | 0.975 |
| 9- IM | 0.800 | 54.202 | 0.054 | 0.989 |
| 9- MM | 0.788 | 56.053 | 0.051 | 0.986 |
| 9- IS | 0.736 | 56.776 | 0.05 | 0.985 |
| 9- MS | 0.815 | 52.215 | 0.058 | 0.992 |

Note: 6=maize seedling stage (soybean seedling stage) samples; 7=maize jointing stage (soybean seedling stage) samples; 8=maize tasseling stage (soybean flowering and podding stage) samples; 9=maize ripening stages (soybean seed expanding stage) samples. IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean). K (the asymptote), s (the time when y =K/2), p (the exponential rate of AWCD change), $r^2$ (correlation coefficient)

intercropping at maize tasseling stage (Fig. 4). At $AWCD_{48h}$, the differences between the samples were largely determined by their utilization of carbonic acids (76.3% of total variability). Carbohydrates, amines, and esters were mainly utilized by IM microbes. Polymers were mainly utilized by IS microbes. At the later time point, $AWCD_{120h}$ differences between the four samples were due to differential utilization of carbohydrates with phosphate rest, esters, polymers, amino acids, and amines. Polymers were still best metabolized by IM microbial communities. Carbohydrates were utilized equally well by MM and IM soil microbes, as the arrow show between these two samples. Amino acids and amines were metabolized equally well by IM and IS soil microbes. Means intercropping enhances the ability of soil microorganisms to utilize nitrogenous substrate. None of the investigated substrate classes was utilized very well by the MS and MM groups (*i.e.*, the monocropping samples), which showed large differences from the intercropping samples (Fig. 3). Apparently, the MS horizon was located in an isolated position for both AWCD time points, because MS microbes did not utilize any of the investigated substrate classes well and these samples had a unique substrate utilization pattern.

### Utilization of Selected Substrates at $AWCD_{48h}$ and $AWCD_{120h}$ of Maize Tasseling Stage

PCA analysis showed that, at maize tasseling stage (soybean flowering and podding stage), the patterns of carbon source utilization were distinctly separated into intercropping and monocropping groups. Therefore, we conducted a more detailed analysis of the seven groups of carbon substrates at $AWCD_{48h}$ and $AWCD_{120h}$ (Fig. 5) of maize tasseling stage.

For $AWCD_{48h}$, most of the carbon sources were metabolized significantly more by IM microbes than by MM microbes, including CH, CA, AM and ES. Among these, CH MetGlu (β-methyl-D-glucoside), Man (D-mannitol), AceGluc (N-acetyl-D-glucosamine) CA GalLa (D-galactonic acid g-lactone), Gal (D-galacturonic acid), AM Put (Putrescine), and ES PyrMetEs (Pyruvic Acid Methyl Ester) were utilized more efficiently by IM than by MM microbes, and they were utilized more efficiently by MS than by IS microbes.

For $AWCD_{120h}$, the microbial population in the test wells grew adaptably and selectively. The following carbon sources were significantly different between the four samples: CHP, ES, AA, PM and AM (Fig. 4). IM soil microbes showed the highest utilization rate of all ES carbon sources, AA Phe (L-phenylalanine), and PM CycDex (α-Cyclodextrin). Based on this observation, we conclude that the IM microbial specialists are capable of metabolizing these difficult-to-decompose substrates. CHP GluPho (Glucose-1-phosphate), ES Twe80 (Tween80), AA Arg (L-arginine), Thr (L-threonine), AM PheAmi (Phenylethyl-amine), and Put (Putrescine), which are mostly nitrogen-containing compounds, were more frequently utilized by intercropping samples. Moreover, IM soil microbes better metabolized GluPho (Glucose-1-phosphate) and GlyPho (D, L-α-Glycerol-phosphate) than MM soil microbes.

### Microbial Metabolic Diversity Analysis

The results of AWCD120 h analysis showed that the OD of IM 0.94, 0.02 (M, SD) was significantly higher than MM 0.82, 0.03 (M, SD) at maize tasseling stage (soybean flowering and podding stage) ($P < 0.05$). To further investigate the catabolic diversity among different treatments, Shannon's diversity index (H), substrate richness (S), substrate evenness (E), and Simpson's diversity index (J) after 120 h of incubation were estimated for the samples collected at maize tasseling stage (soybean flowering and podding stage) (Table 3). In general, diversity (H, J), richness, and evenness were significantly higher in IM samples than in MM samples. However, for soybean samples, only diversity (H) was significantly increased by intercropping; diversity was higher in the IS condition relative to MS. No significant difference was found between the MM and MS samples collected at maize tasseling stage (soybean flowering and podding stage) ($P > 0.05$). This result indicates that intercropping maize increases microbial communities' substrate utilization (catabolic potential) and functional diversity compared to monocropping.

## Discussion

In this study, we investigated the effect of intercropping on soil physicochemical properties. Our results showed that intercropping maize and soybeans significantly increased

**Table 3:** Effect of intercropping of maize and soybean on soil microbial community catabolic diversity at maize tasseling stage

| Treatment | J | H | E | S |
|---|---|---|---|---|
| IM | 0.961 (0.000)a | 4.762 (0.014)a | 0.977 (0.004)a | 29.333 (0.471)a |
| IS | 0.960 (0.000)ab | 4.732 (0.002)b | 0.961 (0.005)ab | 30.333 (0.471)a |
| MM | 0.958 (0.001)b | 4.685 (0.008)c | 0.958 (0.005)b | 29.667 (0.471)a |
| MS | 0.959 (0.001)ab | 4.697 (0.020)c | 0.964 (0.013)ab | 29.333 (0.943)a |

Note: IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean). J (the Simpson's diversity index); H (Shannon's diversity index); S (substrate richness) and E (substrate evenness). Standard deviations are given in parentheses. Values within the same column not followed by the same letter differ significantly ($P < 0.05$)

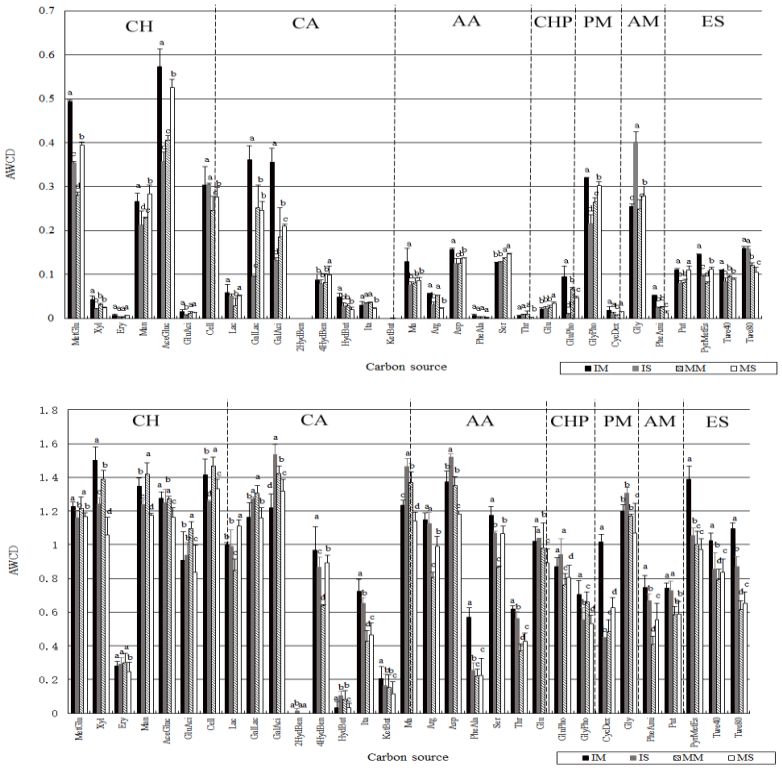


**Fig. 5:** OD value at maize tasseling stage (soybean flowering and podding stage) of $AWCD_{48h}$ (up) and $AWCD_{120h}$ (down)
IM (intercropping maize); MM (monoculture maize); IS (intercropping soybean); MS (monoculture soybean). Different letters indicate significant differences at $P < 0.05$
Carbohydrates without phosphate rest (CH): MetGlu (b-methyl- D -glucoside), Xyl (D-xylose), Ery (i-erythritol), Man (D-mannitol), AceGluc (N-acetyl-D-glucosamine), GluAci (D-glucosaminic Acid), Cell (D-cellobiose), and Lac (a-D-lactose). Carbonic acids (CA): GalLa (D-galactonic acid g-lactone), Gal (D-galacturonic acid), 2HydBen (2-hydroxy benzoic acid), 4HydBen (4-hydroxy benzoic acid), HydBut (g-hydroxy butyric acid), Ita (itaconic acid), KetBut (a-ketobutyric acid), and Ma (D-malic acid). Amino acids (AA): Arg (L-arginine), Asp (L-asparagine), PheAla (L-phenylalanine), Ser (L-serine), Thr (L-threonine), and Glu (glycyl-L-glutamic acid). Carbohydrates with phosphate rest (CHP): GluPho (Glucose-1-phosphate), GlyPho (D,L-α-Glycerol-phosphate). Polymers (PM): CycDex (α-Cyclodextrin), Gly (Glycogen). Amines (AM): PheAmi (Phenylethyl-amine), Put (Putrescine). Ester (ES): PyrMetEs (Pyruvic Acid Methyl Ester), Twe40 (Tween40), Twe80 (Tween80)

nitrate nitrogen ($NO_3^-$-N) content (Fig. 1) and, to some extent, ammonium nitrogen ($NH_4^+$-N) content surrounding the roots of maize. However, when Regehr *et al*. (2015) used $^{15}N$ isotopic pool dilution to study nitrogen content, the authors found that intercropping maize and soybeans significantly increased the gross N immobilization and that soil $NH_4^+$-N was significantly higher in the intercropped maize.

It is possible that differences in analytical techniques or sampling methods between the two studies led to different results. Therefore, more detailed research into the effects of intercropping maize and soybean on the physicochemical properties of soil is needed. Overall, our research suggested that intercropping maize and soybeans significantly increased the available nitrogen in the root-zone of maize. Many studies have shown that intercropped maize has significantly increased nitrogen content (Eaglesham *et al*., 1981; Zhang and Li, 2003). Our results agree with these conclusions from a different perspective.

The AWCD values can reflect the number of soil microorganism species and their size (Harch and Meech, 1997). During the tasseling stage for maize, we observed

significantly higher AWCD values in IM samples 0.94, 0.02 (M, SD) than MM samples 0.82, 0.03 (M, SD) at the 120 h incubation time point ($P < 0.05$), which may imply that the microbial communities in the root-zone soil of intercropped maize are comprised of a different combination of species.

In this study, we augmented CLPP community analysis with analysis of kinetic parameters. This approach gives a more detailed understanding of the nature of the color response (Garland *et al.*, 2001). The time-course of AWCD was modified by the Gompertz equation (O'Connell and Garland, 2002) and the logistic equation (Salomo *et al*., 2009). We used a common multivariate growth model, with three parameters in an S shape, to compare the CLPP of the AWCD across samples. The result showed at maize and soybean seedling stage, all samples had the highest utilization rate of the carbon sources, perhaps because plants need more nutrition in the seedling stage. This finding is consistent with a previous study, which showed that the highest utilization of several monosaccharides by PCA (Folman *et al*., 2001). Because the BIOLOG culturable community is an important contributor to decomposition and enzyme production (Bending *et al*., 2002), and because greater AWCD values indicate that microbial communities use more substrates and have higher activity (Haack *et al*., 1995), we can conclude from our results that maize-soybean intercropping significantly increased the metabolic activity of soil microbes around intercropped maize. Li *et al*. (2012) found that mulberry-soybean intercropping also significantly increased the metabolic activity of intercropping soil microbes using the CLPP method. Intercropping of peanuts with *Atractylodes lancea* can effectively increase soil urease and invertase activity (Dai *et al*., 2013). Together, these studies suggest that intercropping can improve the metabolic activity of soil microbes and the utilization rate of the carbon sources.

At maize tasseling stage (soybean flowering and podding stage), principal component analysis of AWCD at 48 h and 120 h of incubation could clearly separate the soil samples according to their cropping pattern.

At $AWCD_{48h}$, most of the carbon sources were utilized more efficiently by IM soil microbes than by MM microbes, and they were utilized more efficiently by MS than by IS microbes. This may explain, to some extent, why maize is a dominant crop in maize-soybean intercropping, except in the absence of fertilizer (Muyayabantu *et al*., 2013), and why soybean yields are usually 22% lower in the presence of maize. However, the overall productivity of intercropping, as assessed by ATER (area-time equivalent ratio), is significantly higher than monocropping (Clément *et al*., 1992). Additionally, CH Cell (D-cellobiose) and ES Twe80 (Tween80) carbon sources are very different and are all metabolized more efficiently by intercropping crops than monocropping crops.

At $AWCD_{120h}$, we found that intercropping maize improved microbes' ability to decompose phosphorus compounds, such as GluPho and GlyPho. This supports findings that the interspecific stimulation of P uptake may be a general phenomenon, *i.e*., controlled by soil P availability (He *et al*., 2013). We also found that the biochemically inert compound 2HydBen (2-hydroxy benzoic acid, also known as salicylic acid) showed almost no oxidation at all in any of the soil samples, either at $AWCD_{48h}$ or at $AWCD_{120h}$, while the isomeric 4HydBen could be utilized, to a small extent, by all samples.

Although CLPP is more sensitive to changes in the environment (Johnson *et al*., 1998), it is less time consuming and requires less specialized expertise than classic cell-culturing techniques and molecular-level RNA amplification (Campbell *et al*., 2003). However, it is important to note that not all of the 31 substrates in Biolog ECO plate occur in nature, such as CAD-malic acid, or are metabolized by soil microbes. Therefore, future experiments should use custom-prepared plates containing a self-selected combination of carbon sources. In addition, CLPP technology only examines aerobic bacteria out of the many culturable microorganisms that contribute to functional diversity. Therefore, to minimize technical bias, multiple microbiological techniques should be employed to assess microbial communities.

## Conclusion

We observed the dynamics of soil microbe metabolic function diversity in root-zone of maize-soybean intercropping. At the seeding stage of maize the C substrate utilization profiles with PCA are separated based on the crop type (maize or soybean); however, in the tasseling stage of maize, samples are separated based on their intercropping versus monocropping treatment. This suggest that the intercropping become the main factor in the carbon-utilisation profiles of soil microbe in the tasseling stage of maize. We anticipate that our results provide valuable information for the predicting the response of microbial functions to planting time in intercropping system. In addition, our results indicate that at the maize tasseling stage, the M//S enhanced the C-substrate-utilization profiles in maize, leading to more efficient metabolism of nitrogen compounds (such as amine) and other substrates that are typically resistant (such as ester) to degradation in maize root-zone soil. The Shannon's diversity index, Simpson's diversity index, richness, and evenness were significantly higher in IM samples than in MM samples. We provide that the maize is a dominant crop in maize-soybean intercropping from the point of view of soil microorganism function.

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