Effects of Sugarcane-Maize Intercropping on the Metabolic Functional Diversity of Sugarcane Rhizosphere Microorganisms (Postprint)

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Date: 2017-11-07T00:00:00+00:00

Abstract

To investigate the effects of sugarcane-maize intercropping on the functional diversity of sugarcane rhizosphere soil microbial communities, field plot experiments of sugarcane-maize intercropping and sugarcane monoculture were established in Yuanjiang and Longchuan sugarcane planting areas of Yunnan Province, and the Biolog technique was employed to study the effects of sugarcane-maize intercropping on the metabolic functions of sugarcane rhizosphere microorganisms. The results showed that at the Longchuan experimental site, the soil microbial metabolic functional diversity indices—Shannon diversity index, Simpson index, McIntosh index, Shannon evenness index, and McIntosh evenness index—in sugarcane-maize intercropped fields increased by 7.08%, 11.25%, 63.16%, 1.31%, and 2.26%, respectively, compared with sugarcane monoculture fields; at the Yuanjiang experimental site, these indices increased by 10.58%, 48.40%, 43.42%, 0.20%, and 1.65%, respectively. These results indicate that sugarcane-maize intercropping enhanced the diversity of sugarcane rhizosphere soil microorganisms. Sugarcane-maize intercropping increased the utilization efficiency of carbon sources by sugarcane rhizosphere microorganisms, and the carbon source utilization efficiency of sugarcane rhizosphere soil microorganisms in Yuanjiang was lower than that in Longchuan. Compared with monoculture sugarcane, the utilization rates of carbohydrates, amino acids, polymers, amines, carboxylic acids, and phenolic acids in the rhizosphere of intercropped sugarcane at the Yuanjiang experimental site increased by 141.71%, 50.53%, 62.38%, 92.82%, 43.21%, and 6.30%, respectively, while at the Longchuan experimental site they increased by 42.90%, 51.50%, 33.30%, 42.64%, 16.72%, and 24.47%, respectively. Among these, the utilization rates of carbon sources D-cellobiose, D,L-α-glycerol, D-galactono-γ-lactone, L-serine, glycyl-L-glutamic acid, and 2-hydroxybenzoic acid by sugarcane rhizosphere soil microorganisms in intercropped fields at both experimental sites were significantly increased by over 100% compared with monoculture. Through principal component analysis, it was found that sugarcane-maize intercropping altered the community composition and metabolic functions of sugarcane rhizosphere microorganisms, and the three carbon source categories—carbohydrates, carboxylic acids, and amino acids—were sensitive carbon sources that distinguished differences between sugarcane monoculture and intercropping treatments. At the Longchuan experimental site, carbon sources that exerted greater influence on soil microbial metabolic functions mainly included 2 types of polymeric compounds, 6 types of carbohydrates, 2 types of carboxylic acid compounds, 4 types of amino acids, and 1 type of phenolic acid; at the Yuanjiang experimental site, they mainly included 1 type of polymeric compound, 5 types of carbohydrates, 3 types of carboxylic acid compounds, 4 types of amino acids, and 2 types of amine compounds. In summary, ratoon sugarcane-maize intercropping enhanced the diversity of sugarcane rhizosphere microbial communities, increased rhizosphere microbial activity, and altered the metabolic functions of microbial communities. The research findings may provide new insights and a theoretical basis for studies on the effects of crop intercropping on soil microbial diversity analysis and yield-increase mechanisms.

Full Text

Effects of Sugarcane and Maize Intercropping on Sugarcane Rhizosphere Microbe Metabolic Function Diversity

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Abstract: Sugarcane-maize intercropping is an important planting pattern in sugarcane production areas that enhances production benefits, land use efficiency, and pesticide control efficacy. However, the effects of this intercropping system on sugarcane rhizosphere microbial communities remain poorly documented. This study investigated microbial activities, metabolic functional diversity, and carbon source utilization in sugarcane fields in Yuanjiang and Longchuan Counties, Yunnan Province, using Biolog techniques. Compared with monocultured sugarcane at the Longchuan site, the Shannon, Simpson, and McIntosh diversity indices, along with Shannon and McIntosh evenness indices, of rhizosphere microbial communities in intercropped sugarcane increased by 7.08%, 11.25%, 63.16%, 1.31%, and 2.26%, respectively. At the Yuanjiang site, these indices increased by 10.58%, 48.40%, 43.42%, 0.20%, and 1.65%, respectively, compared with sugarcane monoculture. These results demonstrate that intercropping enhanced the metabolic functional diversity of rhizosphere soil microbes. Although carbon resource utilization increased under intercropping, it remained lower at Yuanjiang than at Longchuan. Specifically, compared with monoculture, the Yuanjiang intercropping site showed increased utilization of carbohydrates (141.71%), amino acids (50.53%), polymers (62.38%), amines (92.82%), carboxylic acids (43.21%), and phenolic acids (6.30%). Corresponding increases at Longchuan were 42.90%, 51.50%, 33.30%, 42.64%, 16.72%, and 24.47%, respectively. Notably, utilization of D-cellobiose, D,L-α-glycerol phosphate, D-galactonic acid-γ-lactone, L-serine, glycyl-L-glutamine, and 2-hydroxybenzoic acid increased by over 100% at both sites. Principal component analysis revealed that intercropping altered rhizosphere microbial community composition and metabolic function, with carbohydrates, carboxylic acids, and amino acids serving as the most sensitive carbon sources distinguishing between monoculture and intercropping treatments. At Longchuan, carbon sources significantly influencing microbial metabolic function included two polymers, six carbohydrates, two carboxylic acids, four amino acids, and one phenolic acid. At Yuanjiang, fifteen carbon sources showed significant effects, comprising one polymer, five carbohydrates, three carboxylic acids, four amino acids, and two amines. In conclusion, sugarcane-maize intercropping increased microbial community diversity, activity, and metabolic function in rhizosphere soil, providing new insights and a theoretical foundation for analyzing how crop intercropping affects soil microbial diversity and yield mechanisms.

Keywords: Sugarcane and maize intercropping; Rhizosphere soil; Microbial community; Metabolic function

Soil microorganisms are essential components of soil ecosystems that participate in organic matter decomposition and nutrient transformation, serving as important indicators for soil nutrient status and quality assessment. Soil microbial diversity reflects the comprehensive performance of soil ecosystem structure and function, indicating the intensity of material metabolism and the mutual adaptability between soil environment and microbial communities. Different cultivation methods and crops exert varying effects on soil microbial communities, with appropriate agricultural practices positively regulating microbial community structure and metabolic functional diversity. Conversely, long-term continuous cropping reduces soil microbial biomass, alters microbial diversity, and disrupts community structure balance. Intercropping can significantly increase both soil microbial quantity and diversity indices, particularly in terms of carbon source utilization by soil microorganisms.

Sugarcane (*Saccharum officinarum*) is a globally important sugar crop and energy crop. Continuous ratoon cropping of sugarcane can lead to decreased total rhizosphere microbial biomass and enzyme activity. Intercropping sugarcane with soybean (*Glycine max*) or peanut (*Arachis hypogaea*) can enhance soil microbial activity and enzyme function, while ratoon sugarcane intercropped with soybean can significantly improve rhizosphere soil microflora. Appropriate density intercropping of sugarcane and maize (*Zea mays*) can improve economic benefits, land use efficiency, sugarcane yield, and other agronomic traits while effectively controlling *Ostrinia nubilalis* and other sugarcane pests, making it a widely adopted planting pattern in many sugarcane production regions worldwide. Suman et al. found that sugarcane-maize intercropping increased soil organic carbon content by 25% and soil microbial respiration rate by 42%. However, research on how this intercropping system affects rhizosphere microbial community diversity and function remains scarce.

Studies on how crop diversity planting affects soil microbial structure and function have become a research hotspot in recent years concerning soil fertility protection and ecological effects of crop diversity. This study, conducted in the dry-hot valley sugarcane region of southern Yunnan and the southern sugarcane planting area, employed Biolog technology to investigate the functional diversity of microbial communities in sugarcane rhizosphere soil when intercropped with maize, aiming to provide scientific evidence for protecting farmland ecosystems and establishing rational sugarcane-maize diversity planting systems.

1.1 Experimental Design

The experiment was conducted at two locations: the sugarcane planting area in Lameng Village, Zhangfeng Town, Longchuan County, Dehong Prefecture, Yunnan Province (N24°28 20 , E98°01 45), and the sugarcane planting base at Hongguang Farm, Yuanjiang County, Yuxi City, Yunnan Province (N23°36 26.1 , E101°57 23.4). Longchuan County, located in western Yunnan, features a south Asian tropical monsoon climate with abundant rainfall, ample sunshine, and rich heat resources, showing indistinct seasons with an average annual temperature of 18.9°C. Yuanjiang County, situated in central-southern Yunnan on a low-latitude plateau, has a monsoon climate with average annual temperatures of 12–24°C, warm winters and hot summers, dry and windy conditions in winter and spring, and rainy, humid conditions in summer and autumn, with distinct dry and wet seasons and concurrent rainfall and heat.

The experiment employed a single-factor randomized block design with two treatments: sugarcane monoculture and sugarcane-maize intercropping, each replicated three times with 200 m^2 plots. The sugarcane variety was ‘Yuetang 93-159’ planted as one-year ratoon crop, and the maize variety was ‘Huidan 4’ . Sugarcane was planted in wide-narrow rows with 110 cm wide rows, 70 cm narrow rows, and 20 cm plant spacing. In the intercropping treatment, one row of maize was sown in the wide sugarcane rows at 20 cm plant spacing. The monoculture sugarcane followed the same wide-narrow row pattern. Based on local production practices, maize was planted on February 15 at Yuanjiang and February 25 at Longchuan, with an intercropping duration of 180 days.

1.2 Soil Sampling

Rhizosphere soil samples were collected from sugarcane in both intercropped and monoculture plots on September 16, 2014 (Longchuan) and September 28, 2014 (Yuanjiang). In each plot, samples were taken using a Z-shaped five-point method, with two plants sampled per point. The shaking method was used to collect rhizosphere soil, and samples were combined using the quartering method before being sealed in sterile bags and immediately transported to the laboratory.

1.3 Soil Microbial Activity Measurement

Biolog ECO microplate analysis can reflect the relationship between rhizosphere microbial changes and root exudate utilization. This study used Biolog-ECO plates (31 carbon sources) to analyze soil microbial metabolic functional diversity. Ten grams of soil were added to 90 mL of sterile physiological saline (0.85%), shaken for 30 minutes, left to stand at 4°C for 10 minutes, and the supernatant was diluted 1,000-fold. One hundred fifty microliters of the diluted solution were inoculated into each microplate well and incubated at 25°C. Optical density at 590 nm was measured at 4, 12, 24, 48, 72, 96, 120, 144, 168, and 192 hours using a Biolog Emax™ automated plate reader with Biolog Reader 4.2 software (Biolog, Hayward, CA, USA).

1.4 Data Analysis

Average well color development (AWCD) reflects the overall carbon source utilization capacity of soil microorganisms and serves as an important indicator of microbial activity, with higher values indicating stronger activity. The optical density values at 590 nm were corrected by subtracting the blank control well values, and AWCD from 31 wells was used as an effective indicator of overall activity. Data from 96 hours of incubation were used to calculate microbial diversity indices, carbon source utilization analysis, and principal component analysis (PCA). PCA followed the method of Mao et al., using MultiBase 2015 macros loaded in Microsoft Office Excel 2010, with variable and sample options set for data classification and graphical output. Heatmaps were generated using MetaboAnalyst 3.0 online platform with Euclidean distance for sample clustering. AWCD values, diversity indices, and carbon source utilization rates were processed using Microsoft Excel 2010, and significant differences were analyzed using SPSS 20.0 ($\alpha = 0.05$).

2.1 Effects of Intercropping on Sugarcane Rhizosphere Soil Microbial Activity

As shown in [Figure 1: see original paper], AWCD values showed minimal change during 4–24 hours, increased sharply during 24–96 hours, and remained relatively stable after 120 hours. At 96 hours of incubation, AWCD values in intercropped sugarcane at Longchuan increased by 74.08% compared with monoculture (F =

0.918, $P > 0.05$), while at Yuanjiang, AWCD values increased by 74.68% ($F = 0.238$, $P > 0.05$). Throughout the incubation period, AWCD values in monoculture sugarcane rhizosphere were consistently lower than those in intercropped sugarcane at both sites after 24 hours, indicating that sugarcane-maize intercropping enhanced rhizosphere soil microbial activity.

2.2 Effects of Intercropping on Sugarcane Rhizosphere Soil Microbial Diversity Indices

Microbial metabolic functional diversity indices were calculated from 96-hour incubation data (Table 1). At Longchuan, Shannon, Simpson, McIntosh indices, and McIntosh evenness in intercropped sugarcane were significantly higher than in monoculture ($F = 5.907$, $P = 0.001$; $F = 1.083$, $P = 0.006$; $F = 2.646$, $P = 0.007$; $F = 0.998$, $P = 0.002$), while Shannon evenness showed no significant difference ($F = 1.247$, $P = 0.055$). Similar results were observed at Yuanjiang, where Shannon, Simpson, McIntosh indices, and McIntosh evenness were significantly higher in intercropped sugarcane ($F = 0.031$, $P = 0.001$; $F = 3.866$, $P = 0.001$; $F = 2.317$, $P = 0.002$; $F = 0.01$, $P = 0.048$), with no significant difference in Shannon evenness ($F = 2.067$, $P = 0.932$). At Longchuan, intercropping increased Shannon diversity, Shannon evenness, Simpson index, McIntosh index, and McIntosh evenness by 7.08%, 1.31%, 11.25%, 63.16%, and 2.26%, respectively. At Yuanjiang, these indices increased by 10.58%, 0.20%, 48.40%, 43.42%, and 1.65%, respectively. These results demonstrate that sugarcane-maize intercropping enhanced rhizosphere soil microbial diversity.

2.3 Effects of Intercropping on Carbon Source Utilization by Sugarcane Rhizosphere Soil Microorganisms

Intercropping increased carbon source utilization efficiency by sugarcane rhizosphere microorganisms. As shown in [Figure 2: see original paper], soil microbial carbon source utilization patterns from three replicate plots at both Yuanjiang and Longchuan clustered separately between monoculture and intercropping treatments. Yuanjiang intercropped sugarcane rhizosphere microorganisms clustered with both monoculture and intercropped samples from Longchuan, while Yuanjiang monoculture formed a separate cluster. At both sites, carbon source utilization was lower in monoculture than in intercropped sugarcane, though the magnitude of improvement differed between sites, with overall lower utilization efficiency observed at Yuanjiang.

Analysis of specific carbon source utilization revealed that at Yuanjiang, intercropping significantly enhanced utilization of carbohydrates, amino acids, polymers, and amines ($F = 0.879$, $P = 0.003$; $F = 4.665$, $P = 0.001$; $F = 8.34$, $P = 0.009$; $F = 1.373$, $P = 0$), increasing by 141.71%, 50.53%, 62.38%, and 92.82%, respectively. Carboxylic acids and phenolic acids increased by 43.21% and 6.30%, respectively, but not significantly ($F = 0.005$, $P = 0.082$; $F = 1.083$, $P = 0.729$). At Longchuan, intercropping significantly increased utilization of carbohydrates, amino acids, carboxylic acids, and polymers ($F = 0.263$, $P =$

0.001; F = 6.365, P = 0.013; F = 1.187, P = 0.013; F = 2.546, P = 0.001), with increases of 42.90%, 51.50%, 33.30%, and 42.64%, respectively. Amines and phenolic acids increased by 16.72% and 24.47%, respectively, but not significantly (F = 1.023, P = 0.358; F = 5.973, P = 0.242). Notably, at both sites, utilization of D-cellobiose, D,L-α-glycerol phosphate, D-galactonic acid-γ-lactone, L-serine, glycyl-L-glutamine, and 2-hydroxybenzoic acid increased by over 100% in intercropped plots compared with monoculture.

2.4 Principal Component Analysis of Sugarcane Rhizosphere Soil Microbial Community Structure

PCA based on 96-hour incubation data is presented in [Figure 3: see original paper]. At Longchuan, principal component 1 (PC1) accounted for 57.3% of variation and PC2 for 17.3%, with a cumulative contribution of 74.6%. At Yuanjiang, PC1 accounted for 60.6% and PC2 for 18.9%, totaling 79.5%. Thus, PC1 and PC2 captured most of the variation. Monoculture and intercropped sugarcane were separated along PC1, with monoculture samples in quadrant 2 and intercropped samples in quadrants 1 and 4 at Longchuan, while at Yuanjiang, monoculture occupied quadrants 2 and 3 and intercropping occupied quadrant 1. These results indicate that intercropping altered rhizosphere soil microbial community structure.

Following Choi et al.' s criteria, carbon sources with absolute loading values greater than 0.18 significantly influenced principal components. As shown in , at Longchuan, 15 carbon sources strongly influenced PC1, including two polymers, six carbohydrates, two carboxylic acids, four amino acids, and one phenolic acid (negatively correlated). Ten sources influenced PC2, comprising three carbohydrates (all negatively correlated), one phenolic acid (negatively correlated), three carboxylic acids (α-ketobutyric acid and γ-hydroxybutyric acid negatively correlated, itaconic acid positively correlated), two amino acids (L-threonine positively correlated, glycyl-L-glutamine significantly negatively correlated), and one amine (negatively correlated). At Yuanjiang, 15 carbon sources influenced PC1, including one polymer, five carbohydrates, three carboxylic acids (α-ketobutyric acid negatively correlated, others positively correlated), four amino acids, and two amines. Twelve sources influenced PC2, comprising one polymer, two carbohydrates, five carboxylic acids (pyruvic acid methyl ester negatively correlated, others positively correlated), three amino acids (L-asparagine positively correlated, L-threonine and L-phenylalanine negatively correlated), and two phenolic acids. These results indicate that carbohydrates, carboxylic acids, and amino acids are sensitive carbon sources for distinguishing microbial metabolic differences between monoculture and intercropping treatments.

3.1 Sugarcane-Maize Intercropping Enhanced Rhizosphere Soil Microbial Activity

AWCD serves as a crucial indicator of soil microbial metabolic activity. This study demonstrated that intercropping increased rhizosphere soil microbial activity by over 74% at both Longchuan and Yuanjiang, consistent with results from sugarcane/peanut and sugarcane/soybean intercropping systems. These findings indicate that sugarcane-maize intercropping significantly improves rhizosphere soil microbial activity, thereby demonstrating considerable yield enhancement potential.

3.2 Effects of Intercropping on Rhizosphere Microbial Diversity

Appropriate intercropping can enhance soil microbial diversity. Using Biolog technology, this study found that sugarcane intercropping with maize increased soil microbial diversity indices, AWCD values, and utilization of 31 carbon sources compared with monoculture, corroborating previous reports on sugarcane-soybean intercropping. PCA revealed that intercropping markedly altered rhizosphere microbial community functional diversity, primarily through differences in carbon source utilization. At Longchuan, highly utilized carbon sources included six carbohydrates, two carboxylic acids, and four amino acids, while at Yuanjiang, they comprised five carbohydrates, three carboxylic acids, and four amino acids. This indicates that carbohydrates, amino acids, and carboxylic acids are sensitive carbon sources distinguishing functional diversity between monoculture and intercropping, consistent with findings from wheat/faba bean, rice/watermelon, mulberry/soybean, and konjac/maize intercropping systems. Badri et al. reported that changes in soil microbial diversity can affect crop secondary metabolites, thereby influencing herbivore feeding behavior. Varun et al. and Zhang et al. reported effective pest control in sugarcane-maize intercropping systems. Whether this control effect results from altered soil microbial diversity warrants further investigation.

3.3 Effects of Intercropping on Utilization of Different Carbon Sources by Rhizosphere Microorganisms

This study found that sugarcane-maize intercropping improved utilization of various carbon sources to different extents. At Yuanjiang, average utilization rates for carbohydrates, amino acids, polymers, amines, carboxylic acids, and phenolic acids increased by 141.71%, 50.53%, 62.38%, 92.82%, 43.21%, and 6.30%, respectively, while increases at Longchuan were lower at 42.90%, 51.50%, 33.30%, 42.64%, 16.72%, and 24.47%, respectively. These results demonstrate that intercropping significantly enhanced utilization of phenolic acids, amino acids, and carbohydrates. The differential promotion effects between sites likely resulted from differences in original soil texture and climate.

Notably, 2-hydroxybenzoic acid (salicylic acid), which some plants produce in response to insect herbivory to induce resistance, showed significantly increased utilization in intercropped plots, with increases of 228.93% at Yuanjiang and 105.42% at Longchuan. Since sugarcane-maize intercropping demonstrates effective pest control, whether this effect relates to enhanced microbial utilization of such compounds requires further study. Additionally, utilization of D-cellobiose, D,L-α-glycerol phosphate, D-galactonic acid-γ-lactone, L-serine, glycyl-L-glutamine, and 2-hydroxybenzoic acid increased by over 100% at both sites. Whether these changes in carbon source utilization promote sugarcane yield also merits further investigation.

This study demonstrates that ratoon sugarcane intercropped with maize effectively enhanced rhizosphere soil microbial diversity and activity, altered microbial community structure and metabolic function, and reduced community dysfunction, which is significant for protecting soil microorganisms in sugarcane fields. These findings provide new perspectives and theoretical foundations for analyzing how crop intercropping affects soil microbial diversity and yield mechanisms. Carbohydrates, carboxylic acids, and amino acids emerged as sensitive carbon sources for distinguishing metabolic differences between monoculture and intercropping treatments. However, which specific microbial taxa are altered by sugarcane-maize intercropping and their regulatory roles in sugarcane and maize growth require further investigation.

Acknowledgments

We thank Li Chengzhen from Yuanjiang Agricultural Technique Center, master’ s students Wang Yixing (2010), Liu Huwei and Ning Ge (2011), and undergraduate students Pu Changsheng, Zhu Xiang, Liu Mingfu, Zhang Famin, Hong Dan, and Cheng Xingmeng from Yunnan Agricultural University for their assistance with this research.

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