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RESEARCH ARTICLE

Effects of sugarcane-duck symbiosis on rhizobacterial community structure and interannual variability

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ABSTRACT

Sugarcane-duck symbiosis (SDS) is an ecological breeding production method wherein meat ducks are released in sugarcane (*Saccharum officinarum* L.) fields during the middle and late stages of sugarcane growth. The effects of SDS on sugarcane rhizobacterial diversity remain poorly understood. We conducted field experiments with sugarcane monoculture (SC) and SDS and performed genetic analyses on the rhizobacterial communities based on 16S rRNA across different growth stages of sugarcane. The effects of soil physicochemical factors on the bacterial community structure were analyzed via redundancy analysis. We found that soil organic matter (SOM), total N (TN), nitrate N (NN), and ammonium N (AN) contents of sugarcane rhizosphere soils increased during different growth periods in SDS. During the perennial root stages, the Shannon-, Ace-, and Chao1 indices increased, whereas the available P (AP) content decreased. The soil pH was more balanced and its effect on the soil bacterial community was insignificant. The dominant sugarcane rhizosphere flora were Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, *norank_f__norank_o__Gaiellales*, *Sphingomonas*, *norank_f__Roseiflexaceae*, *Bacillus*, and *norank_f__Xanthobacteraceae*, but with significant inter-annual variations. Redundancy analysis showed that SOM, TN, NN, AN, AP, catalase (CAT) and invertase (INV) activity were closely associated with the diversity of the soil bacterial communities, and TN and INV activity were the main drivers of sugarcane rhizobacterial communities in the SDS groups. In conclusion, SDS improves the fertilizer supply capacity and soil quality of sugarcane soils by regulating major soil nutrient content, available nutrients, and microbial diversity. This study provides a theoretical basis for scientifically managing SDS and the screening of biocontrol bacteria.

Key words: Bacterial community, growth period, meat duck, rhizosphere, sugarcane-duck symbiosis.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is the most important sugar crop and plays an important role in sustaining the stability of the Chinese sugar supply (Verma et al., 2022). Recently, the traditional cultivation model based on sugarcane monoculture has led to a significant increase in the use of pesticides, fertilizers and chemical herbicides. This has resulted in decreased stability and sustainability of farmland ecosystems, and environmental problems such as contaminated soil, with reduced microbial diversity and sugarcane yields that have seriously hindered further development of the Chinese sugarcane industry. Therefore, balancing a high yield and quality of sugarcane with effective utilization of soil nutrients has become a hot topic of agricultural science in recent years. The integrated livestock-crop systems have the effect of improving soil structure and increasing soil fertility (Pontes et al., 2021). Sugarcane-duck symbiosis (SDS) is an ecological production method wherein ducks are released in a sugarcane field at the middle and late

growth stages of sugarcane (Ma et al., 2022a). This approach can utilize the ecological advantages of the mutual beneficial symbiosis between ducks and sugarcane, reduce the dependence on chemical fertilizers and pesticides, and decrease agricultural surface pollution. Sugarcane fields provide ducks with living space and high-quality animal and plant feed. In turn, meat ducks catch pests and remove weeds, and duck manure is returned to the field to moderate soil acidity and fertilize the ground, thus reducing the use of fertilizers and pesticides (Ma et al., 2022b).

Soil bacteria are the most numerous and diverse group of soil microorganisms, accounting for approximately 70%-90% of the total soil microorganisms (Singh et al., 2023). Soil bacteria are the most active biotic factor in the cycling of matter and energy flow in soil ecosystems and play key roles in soil fertility, soil health, and plant growth and development (Yang et al., 2018). Many studies show that the livestock-crop system can improve the physical structure of the soil, increase permeability, reduce soil bulk weight, increase the soil organic matter (SOM), available N, available P (AP), available K, and other nutrients in shallow soil, and improve soil enzyme activity (Wei et al., 2019).

Fine-scale studies were conducted to elucidate the effects of integrated rice farming models on soil physicochemical properties, enzyme activities, and microbial diversity (Guo et al., 2022). However, the effects of SDS as an ecological farming method on the sugarcane rhizobacterial community structure and interannual variability were investigated. In this study, physiochemical properties and rhizobacterial (16S rRNA) diversity of the soil at different sugarcane growth stages were compared between sugarcane monoculture (SC) and SDS cultivation conditions. This study provides a scientific basis for interpreting the effects of SDS on microbial diversity in sugarcane soils, laying a theoretical foundation for the development of specialized fungicides for sugarcane across different developmental stages.

MATERIALS AND METHODS

Plants and field experimental design

The study area was selected based on the location of the ecological breeding base of mackerel ducks in Luohui Village (106°39' E, 22°27' N; 138.7 m a.s.l.), Guangxi, China. The area has a typical southern subtropical monsoon climate, with an annual temperature and rainfall of 22 °C and 1273.6 mm, respectively. The study area has 1547.1 h annual sunshine and is frost-free throughout the year. The experimental area had Ferralsols with deep layers, relatively flat terrain, good drainage and irrigation, soil pH 7.76, 19.93 g kg^{-1} soil organic matter (SOM), 11.56 g kg^{-1} organic C, 3.58 mg kg^{-1} available P, 32 mg kg^{-1} available K, and 14.96 cmol kg^{-1} cation exchange capacity. The sugarcane (*Saccharum officinarum* L.) 'Guinanzhe 146210' was provided by the Guangxi South Subtropical Agricultural Scientific Research Institute (Chongzuo, China). This cultivar has the characteristics of resistance to fall, disease and insect, and lodging, which was suitable for sugarcane-duck symbiosis (SDS) cultivation mode. The duck test variety was Jinding (*Anas platyrhynchos domestica*), with a small size, high quality meat, and strong adaptability suitable for sugarcane-duck symbiosis (SDS) cultivation mode, which was hatched and bred by Longzhou Nongrun Agriculture Co. Ltd. (Chongzuo, China).

Soil sample collection and analysis of physicochemical properties

The field experiment was started in March 2021 using a randomized plot design with two treatments of sugarcane monoculture (SC) and SDS, three replicates for each treatment, and a total of six sugarcane plots, with approximately 1300 m^2 for each plot, which were separated from each other by fences. The SC was planted in wide-narrow (1.8 × 0.6) rows. The planting density was 135 000 buds ha^{-1} and fertilizers were applied once before the planting of sugarcane or at the time of breaking the ridge of the lodging root, and the amount of fertilizers applied was 300.0 kg N ha^{-1}, 120.0 kg P_2O_5 ha^{-1}, and 255.0 kg K_2O ha^{-1}. The planting method, fertilizer application rate, pest and disease management, and related farming operations of the SDS are the same as those of the SC. For SDS, 5000 Jinding ducks (10 d old) were maintained in a greenhouse next to the sugarcane field for 10 d before they were released into the field. The ducks and sugarcane coexisted for 40 d at a density of one bird per square meter. Ducks were fed regularly, and the

dietary nutrition level met the requirements of the agricultural industry standard NY/T 2122-2012 (produced by Guigang Haida Feed Co., Ltd., China). For the SC condition, no ducks were released into the sugarcane field. Both model treatments were free of pesticides and chemical herbicides throughout the sugarcane growth period.

A field orientation trial was conducted from August 2021 to December 2022, and the straw from the sugarcane harvest was returned to the field each year. Soil samples were collected on 12 September 2021: year-one elongation, SC new plant elongation (SCNE) and SDS new plant elongation (SDNE); 5 December 2021: year-one maturity, SC new plant maturity (SCNM) and SDS new plant maturity (SDNM); 2 September 2022: year-two elongation, SC perennial root elongation (SCPE) and SDS perennial root elongation (SDPE); and 25 November 2022: year-two maturity, SC perennial root maturity (SCPM) and SDS perennial root maturity (SDPM). The inter-root soil was collected from a depth of approximately 0 to 20 cm in the soil layer of each plot according to the S-shaped "five-point sampling" method and the "root shaking" method (Wang et al., 2018). Fresh soil samples collected for each treatment were separately removed from impurities and divided into two sections. One portion of the soil sample was immediately wrapped in tin foil and transported back to the laboratory on dry ice before storing at -80 °C for bacterial diversity analysis. The other portion was placed in a sealed bag and used to determine the physiochemical properties of the soil.

The physiochemical properties of the soil were determined according to NY/T 1121-2006 soil testing standards. Soil total N (TN) and total C were determined using an elemental analyzer (ECS4024, Costech Analytical Technologies Inc., Valencia, California, USA). Total P (TP) and available P (AP) levels were determined using the molybdenum antimony colorimetric method. Soil nitrate N (NN) and ammonium N (AN) contents were determined by leaching in a 2 mol L^{-1} KCl solution and using a flow analyzer (AA3; Bran+Luebbe, Norderstedt, Germany). Invertase (INV) activity was determined with a 3,5-dinitrosalicylic acid colorimetric assay and urease (URE) activity was assessed with an indophenol blue colorimetric assay according to the methods described by Gu et al. (2009). 3,5-Dinitrosalicylic acid was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Catalase (CAT) activity was determined using the method described by Trasar-Cepeda et al. (1999).

DNA extraction, high-throughput sequencing, and analysis

Total soil microbial DNA was extracted using the MPBio Soil DNA Extraction Kit (MoBio Laboratories Inc., Carlsbad, California, USA) according to the manufacturer's instructions. The quantity and quality of the extracted DNA were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 0.8% agarose gel electrophoresis, respectively. Polymerase chain reaction (PCR) amplification of the V3-V4 variable region of the bacterial 16S rRNA gene was performed using universal primers 338F (5′-ACTCCTACGGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The library was constructed using the TruSeq PCR-Free Sample Prep Kit (FC-121-3001/3002), according to the manufacturer's instructions, and high-throughput sequencing was performed using the Illumina MiSeq PE300 platform (San Diego, California, USA) by Shanghai Meiji Bio-medical Technology Co., Ltd. (Shanghai, China).

Bioinformatic statistics were performed to generate operational taxonomic unit (OTU) tables, with chao-, Ace-, Shannon-, Simpson-, and Coverage indices (Edgar et al., 2011; Pitta et al., 2014). Graphing, principal coordinate analysis (PCoA), linear discriminant analysis Effect Size (LEfSe), and environmental factor correlation analysis were performed using online R language tools, all of which were completed on the online platform of Majorbio Cloud Platform.

The experimental data were computationally organized and visualized using Excel 2021 and analyzed using one-way ANOVA using SPSS 22.0 (IBM, Armonk, New York, USA). The data were first tested for variance alignment and those with variance alignment were analyzed using two-way ANOVA and compared for the significance of differences between treatments using Duncan's multiple comparison method. Differences with $P < 0.05$ were considered significant and those with $P < 0.01$ were considered highly significant. Data with uneven variance were log-transformed as log (x +1).

RESULTS

Effect of SDS on physiochemical properties of sugarcane rhizosphere soils

The physicochemical properties of sugarcane rhizosphere soils changed during different growth stages between the years after the introduction of ducks (Table 1). In the SDS rhizosphere soils, SOM, TN, pH, and INV activity at SDNM decreased by 5.65%, 11.80%, 20.30%, and 45.18%, respectively, compared to those of the SCNM rhizosphere soils, whereas CAT and URE activities increased by 34.04% and 22.59%, respectively. At the SDNE, TP, NN, AN, and AP increased by 9.91%, 75.42%, 16.01%, and 26.03%, respectively, whereas at the SDNM, they decreased by 16.81%, 194.98%, 141.10%, and 31.10%, respectively, compared to those of the SC sugarcane rhizosphere soils. In the SDS rhizosphere field soils, SOM, TN, NN, and AN in SDPM increased by an average of 9.28%, 4.87%, 98.95%, and 64.94%, whereas AP, pH, CAT and URE activity decreased by an average of 19.69%, 12.44%, and 16.94%, respectively, compared to those of the SCPM rhizosphere soils. During the SDPE period, TP, AN, URE and INV activity increased by 10.38%, 136.54%, 3.03%, and 12.13%, respectively, whereas during the SDPM period, they decreased by 4.99%, 6.67%, 59.52%, and 18.61%, respectively, compared to those of the SC sugarcane rhizosphere soils. These results demonstrated that SDS had a more significant effect on soil improvement in year two than in year one. Soil pH significantly decreased in SDS compared to that of SC. After year-one SDS, SOM and TN decreased, whereas CAT and URE activities increased compared to those of SC. After year-two SDS, SOM, TN, NN, and AN increased, whereas the AP content and CAT and URE activities decreased compared to those of SC.

Table 1. Physicochemical factors of sugarcane rhizosphere soil during different growth periods under the two planting methods. Lowercase letters in the same column indicate the variability in different growth periods in the same sugarcane field, whereas uppercase letters indicate the variability in the same growth period in different sugarcane fields ($P < 0.05$). SCNE: Sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity.

Growth stage	Soil organic matter	Total N	Total P	Nitrate N	Ammonium N	Olsen P	pH	Catalase	Urease	Invertase
	g kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}		μg g^{-1} h^{-1}	μg g^{-1} h^{-1}	μg g^{-1} h^{-1}
SCNE	20.53 ± 0.91^{bA}	1.226 ± 0.056^{bA}	0.678 ± 0.011^{aB}	6.38 ± 0.23^{cB}	3.99 ± 0.06^{aB}	25.4 ± 0.5^{bB}	7.82 ± 0.05^{aA}	4.24 ± 0.12^{bA}	11.85 ± 0.16^{bA}	1444.21 ± 48.03^{aA}
SCNM	24.06 ± 0.26^{aA}	1.346 ± 0.026^{abA}	0.676 ± 0.011^{aA}	18.63 ± 0.59^{aA}	3.56 ± 0.20^{bA}	36.5 ± 0.6^{aA}	7.48 ± 0.10^{bA}	5.92 ± 0.08^{aB}	15.83 ± 0.43^{aB}	215.80 ± 11.96^{bA}
SCPE	18.21 ± 0.14^{cB}	1.295 ± 0.023^{abB}	0.549 ± 0.006^{cB}	6.68 ± 0.18^{cB}	3.53 ± 0.10^{bB}	21.0 ± 0.4^{cA}	6.35 ± 0.10^{cA}	2.32 ± 0.06^{dA}	0.33 ± 0.02^{dA}	11.46 ± 0.12^{cA}
SCPM	22.87 ± 0.79^{abA}	1.426 ± 0.041^{aA}	0.581 ± 0.007^{bA}	8.76 ± 0.19^{bB}	0.90 ± 0.08^{cA}	24.5 ± 0.3^{bA}	8.08 ± 0.02^{aA}	3.75 ± 0.04^{cA}	1.26 ± 0.06^{cA}	17.57 ± 1.10^{cA}
SDNE	19.43 ± 0.11^{cA}	1.045 ± 0.026^{dB}	0.792 ± 0.006^{aA}	18.82 ± 0.45^{aA}	9.62 ± 0.18^{aA}	33.3 ± 0.4^{aA}	5.59 ± 0.03^{cB}	4.09 ± 0.08^{bA}	12.66 ± 0.67^{bA}	1130.86 ± 64.18^{aB}
SDNM	22.63 ± 0.24^{aB}	1.227 ± 0.009^{cB}	0.609 ± 0.008^{bB}	4.58 ± 0.41^{cB}	2.99 ± 0.16^{cA}	27.0 ± 0.8^{bB}	6.57 ± 0.05^{bB}	10.16 ± 0.19^{aA}	21.90 ± 0.47^{aA}	67.62 ± 2.95^{bB}
SDPE	22.72 ± 0.10^{aA}	1.461 ± 0.014^{aA}	0.606 ± 0.005^{bA}	18.71 ± 0.26^{aA}	8.35 ± 0.13^{bA}	17.1 ± 0.3^{dB}	5.58 ± 0.05^{cB}	1.93 ± 0.03^{dB}	0.34 ± 0.02^{cA}	12.85 ± 0.77^{cA}
SDPM	21.45 ± 0.19^{bA}	1.382 ± 0.010^{bA}	0.552 ± 0.005^{cB}	10.32 ± 0.15^{bA}	0.84 ± 0.08^{dA}	19.4 ± 0.4^{cB}	7.05 ± 0.08^{aB}	3.11 ± 0.05^{cB}	0.51 ± 0.04^{cB}	14.30 ± 0.95^{cA}

Influence of SDS on soil bacterial community diversity

A total of 1 280 979 valid sequences were obtained for the 24 samples from the SC and SDS fields. The coverage was greater than 0.94, indicating that the sequencing reached the required depth and covered all species. Using the 97% similarity level as the basis for division, 2713-3914 OTUs were obtained for each stage in SC and SDS (Table 2). The number of OTUs in SC and SDS increased with sugarcane growth, with a significant difference observed in SDS ($P = 0.001$). Analysis of the three α-diversity indices (Shannon, Ace, and Chao1) showed that both cultivation conditions showed an increasing trend of microbial diversity with sugarcane growth ($P < 0.05$). In contrast, the Simpson index showed a decreasing trend with sugarcane growth, and the difference was significant in SC ($P < 0.05$) but insignificant in SDS ($P > 0.05$). The Shannon-, Ace-, and Chao1 indices were reduced by 1.89%, 9.60%, and 6.41%, respectively in the SDNM compared to that of SCNM. In SDS, the Shannon-, Ace-, and Chao1 indices of SDPE increased by 4.01%, 25.77%, and 14.82%, respectively ($P < 0.05$), whereas they decreased by 1.15%, 6.14%, and 6.07%, respectively ($P > 0.05$) in SDPM compared with those detected at the same stage in SC.

Table 2. Statistical analysis of high-throughput sequencing data and α-diversity indices of the soil bacterial community. Lowercase letters in the same column indicate the variability in different growth periods in the same sugarcane field, whereas uppercase letters indicate the variability in the same growth period in different sugarcane fields ($P < 0.05$). SCNE: sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity; OUT: operational taxonomic unit.

	Effective sequence		Diversity index		Abundance index		
Growth stage	numbers	OTU numbers	Shannon	Simpson	Ace	Chao1	Coverage
SCNE	48522 ± 7366	3220 ± 12^{bA}	6.60 ± 0.03^{bA}	0.0046 ± 0.0004^{abA}	5674.37 ± 25.67^{bA}	4804.36 ± 20.45^{bA}	0.96
SCNM	57590 ± 4425	3271 ± 316^{bA}	6.60 ± 0.12^{abA}	0.0051 ± 0.0007^{abA}	6029.40 ± 395.61^{bA}	4937.78 ± 294.00^{bA}	0.95
SCPE	45322 ± 1971	3041 ± 128^{bB}	6.49 ± 0.02^{bB}	0.0053 ± 0.0002^{aA}	4730.90 ± 206.66^{cB}	4404.50 ± 165.69^{bB}	0.96
SCPM	59419 ± 2120	3889 ± 36^{aA}	6.95 ± 0.01^{aA}	0.0031 ± 0.0001^{bA}	7066.39 ± 198.50^{aA}	5888.80 ± 158.23^{aA}	0.94
SDNE	46545 ± 2411	2826 ± 57^{cB}	6.38 ± 0.04^{cB}	0.0051 ± 0.0003^{aA}	4830.93 ± 393.63^{cA}	4328.31 ± 131.70^{cB}	0.96
SDNM	60726 ± 1129	3115 ± 72^{bA}	6.57 ± 0.03^{bcA}	0.0042 ± 0.0003^{aA}	5768.41 ± 104.42^{bA}	4794.25 ± 126.20^{bA}	0.96
SDPE	47596 ± 1360	3332 ± 73^{bA}	6.75 ± 0.07^{abA}	0.0042 ± 0.0006^{aA}	5950.08 ± 64.31^{abA}	5057.41 ± 22.79^{bA}	0.95
SDPM	57375 ± 636	3625 ± 109^{aA}	6.87 ± 0.09^{aA}	0.0033 ± 0.0006^{aA}	6632.86 ± 283.32^{aA}	5531.43 ± 209.57^{aA}	0.95

Changes in soil bacterial community structure and abundance in sugarcane rhizobacteria at different growth stages under SC and SDS cultivation

The sequences obtained from the sugarcane rhizosphere soil at different growth stages in SC and SDS were classified into 36 phyla, 101 classes, 216 orders, 346 families, 630 genera, and 1225 species. Phyla with a relative abundance < 1% were categorized as "Others." The dominant phyla were Actinobacteria (20.50%-40.45%), Proteobacteria (16.94%-29.68%), Chloroflexi (11.75%-20.99%), Acidobacteriota (4.36%-12.27%), and Firmicutes (2.79%-12.12%). The samples also contained Gemmatimonadota, Myxococcota, Bacteroidetes, Methylomirabilota, Patescibacteria, Planctomycetota, and Nitrospirota (Figure 1A). The relative abundance of the five dominant phyla significantly differed between the SC and SDS groups at each

stage (P < 0.05). Actinobacteria showed a downward trend during the sugarcane growth, whereas Proteobacteria and Acidobacteriota showed gradual increases in relative abundance along the sugarcane growth trajectory. The relative abundance of Chloroflexi in the SC field was higher than that in SDS. The relative abundance of Firmicutes in SC increased and then decreased with the sugarcane growth stage, which increased to the highest value in SCPE, whereas the relative abundance tended to increase with the growth stages in SDS. These differences were significant (P < 0.05).

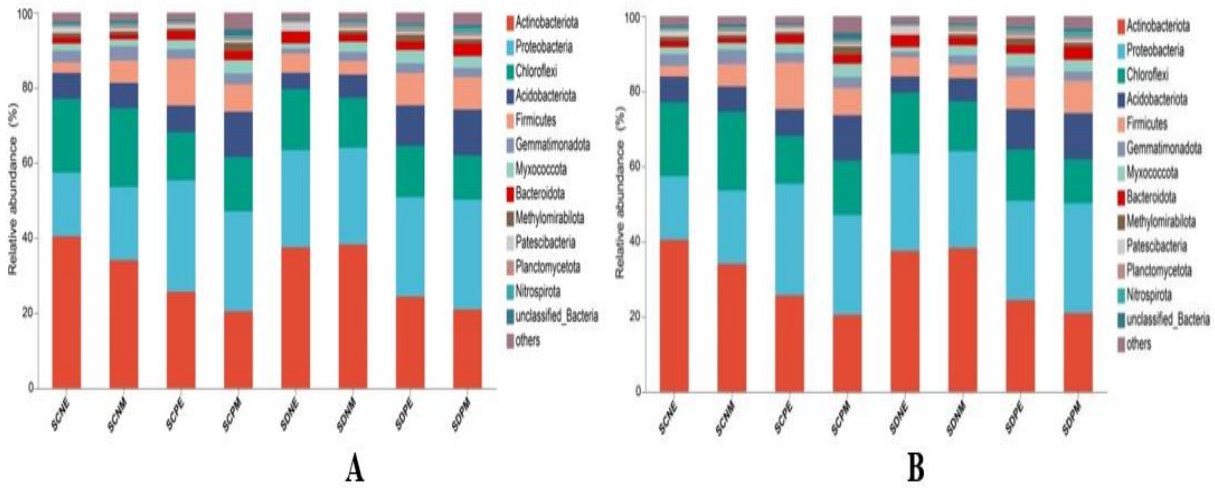

Figure 1. Composition of the rhizobacterial community at the phylum level (A) and the genus level (B) in different growth stages and cultivation conditions. SCNE: Sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity.

A total of 2 and 46 bacterial genera out of the 630 genera identified from the 24 samples had relative abundances greater than 0.5% and 0.1%, respectively (Figure 1B). The most abundant genera were *norank_f__norank_o__Gaiellales*, *Sphingomonas*, *norank_f__Roseiflexaceae*, *Bacillus*, *norank_f__Xanthobacteraceae*, *norank_f__JG30-KF-CM45*, *Nocardioides*, *norank_f__norank_o__Vicinamibacterales*, *Gaiella*, *Bradyrhizobium*, *norank_f__Gemmatimonadaceae*, *norank_f__67-14*, *Acidothermus*, *norank_f__Vicinamibacteraceae*, *Streptomyces*, *norank_f__JG30-KF-AS9*, *Terrabacter*, *norank_f__norank_o__norank_c__TK10*, *Arthrobacter*, and *Micromonospora*. The sum of the relative abundance of these 20 bacterial genera accounted for over 35% of the total abundance of soil bacteria that were dominant throughout the sugarcane growth and development periods.

Beta-diversity of sugarcane rhizobacterial communities at different growth stages

Beta-diversity analysis was used to explore similarities or differences in community composition and to analyze species diversity among microbial communities in a comparative, between-groups analysis. Principal coordinate analysis (PCoA) is a method of beta-diversity analysis in which samples that cluster closer to each other are considered to have a more similar species composition, whereas more distant sample points represent a more significant difference in species composition between samples. There was a clear distance between the growth stages in the SC and SDS fields (Figure 2), indicating that the structure of the sugarcane rhizobacterial communities significantly changed after the introduction of meat ducks into the

sugarcane fields. The first and second coordinate axes explained 35.4% and 25.65% of the total variance, respectively. Significant intergroup differences were found between the bacterial communities of the SC and SDS at different growth stages (R^2 = 0.963, P < 0.05). The proximity of SC and SDS samples on the principal coordinates plot was closer during the perennial root elongation period, suggesting a high similarity of bacterial communities. The remaining three growth stages showed some differences between SC and SDS, indicating that the bacterial communities changed and differentiated during sugarcane growth.

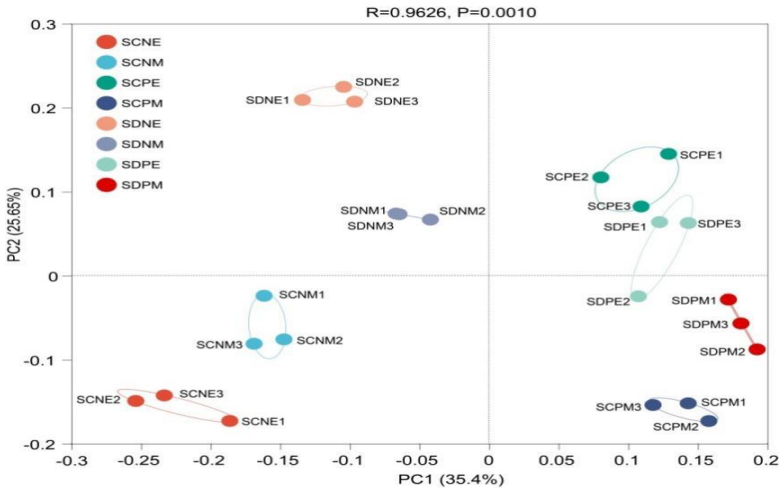

Figure 2. Principal coordinate analysis plot of soil bacterial operational taxonomic units. SCNE: Sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity.

Changes in bacterial flora of sugarcane rhizosphere soil at different growth stages in SC and SDS

Linear discriminant analysis of effect size (LEfSe) can be used to compare soil microbial communities and capture signature microbial species that significantly differ among treatments (Segata et al., 2011). When the linear discriminant analysis score was 4, a total of 12, 6, 8, 8, 17, 4, 4, and 6 OTUs were identified in the rhizosphere soils for SCNE, SCNM, SCPE, SCPM, SDNE, SDNM, SDPE, and SDPM, respectively (Figure 3). Among the SCNE samples, the identified OTUs included the phyla Actinobacteria and Chloroflexi, which included *norank_f__Roseiflexaceae*, *Marmoricola*, *Gaiella*, *norank_f__JG30-KF-CM45*. The OTU identified for SCNM was *Nitrolancea*. The SCPE OTUs were mainly found in the phyla Proteobacteria and Firmicutes and included *Bacillus* and *Bradyrhizobium*. The OTUs for SCPM were in the genus *norank_f__Vicinamibacteraceae*. The OTUs of SDNE were mainly within the phyla Actinobacteria, Proteobacteria, and Firmicutes and included *norank_f__JG30-KF-AS9*, *Sphingomonas*, *Acidothermus*, and *Chujaibacter*. The OTUs for SDNM were mainly found in the phylum Actinobacteria and included *Nocardia*. The OTUs for SDPE were members of the genus *norank_f__Xanthobacteraceae* and those for SDPM were found in the genus *norank_f__norank_o__Vicinamibacterales*. These results demonstrate that the OTUs differed among the eight treatment groups.

Association of rhizosphere community composition and soil physicochemical factors during different growth stages in SC and SDS

The selected environmental factors (SOM, TN, TP, AP, NN, AN, pH, URE, INV, and CAT activity) were screened and those with a variance inflation factor (VIF) less than 10 were retained for subsequent analysis,

including SOM, TN, NN, AN, AP, CAT, and INV. Based on the initial RDA, axes 1 and 2 accounted for 67.04% and 10.72% of the variance, respectively, with 77.76% of the total variance explained (Figure 4A). Actinobacteria were positively correlated with Chloroflexi and negatively correlated with Proteobacteria, Acidobacteria, and Firmicutes. Positive correlations were observed among Proteobacteria, Acidobacteria, and Firmicutes. Actinobacteriota positively correlated with INV (r = 0.7157, P < 0.001), CAT (r = 0.64, P < 0.001), AP (r = 0.5878, P < 0.01), and AN (r = 0.5435, P < 0.01), and negatively correlated with TN (r = -0.7574, P < 0.0001). Proteobacteria negatively correlated with SOM, NN, AP, CAT, and INV and positively correlated with other physicochemical factors, whereas the opposite was found for Chloroflexi. Acidobacteria were highly positively correlated with TN and negatively correlated with AP, INV, CAT, and AN. Firmicutes only showed a highly significant correlation with TN and was negatively correlated with INV, CAT, and AP.

Axis 1 of the RDA-based plot at the genus level explained approximately 26.76% of the variance and axis 2 explained 22.02% of the variance, representing 48.78% of the total variance explained (Figure 4B). SOM, TN, NN, AN, AP, CAT, and INV were the major factors controlling the microbial communities. TN positively correlated with SOM and negatively correlated with NN, AN, AP, CAT, and INV. INV positively correlated with AP, AN, NN, SOM, and CAT and negatively correlated with TN. SOM positively correlated with TN, CAT, and INV and negatively correlated with AP, AN, and NN. Positive correlations were observed among the remaining environmental factors. The SCPM and SDPM showed a strong correlation with TN. The SDPE was closely associated with *Bacillus* and *norank_f__Xanthobacteraceae*, and SDNE was closely associated with AN and *Sphingomonas*. The SCPE was strongly associated with *Bradyrhizobium*.

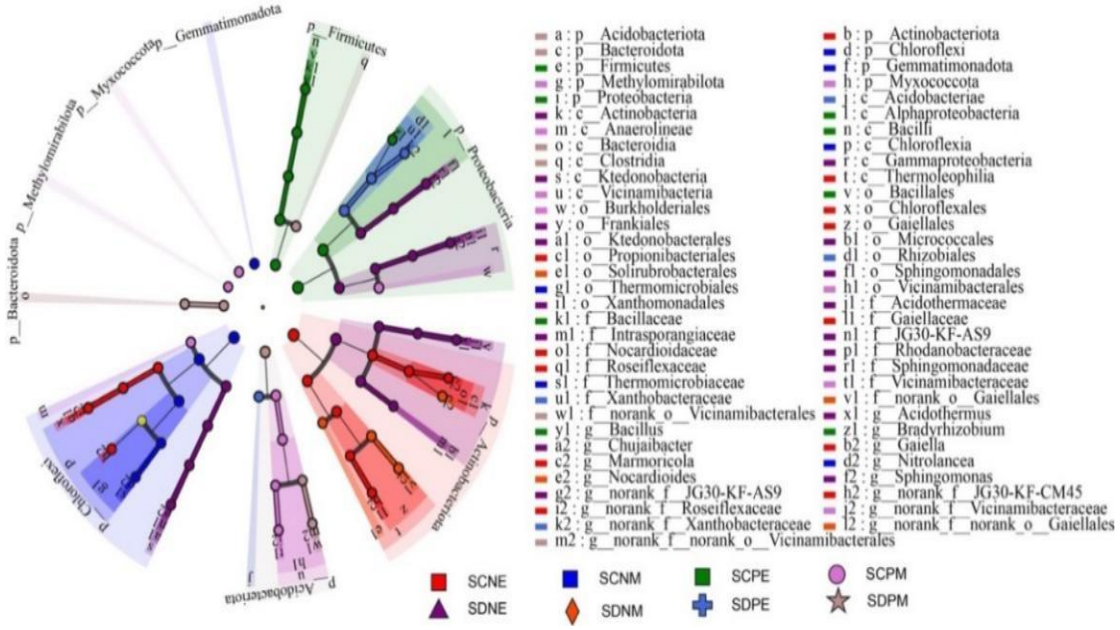

Figure 3. Linear discriminant analysis of effect size of sugarcane rhizobacterial communities under different growth periods in the two cropping models. The groups at the phylum, class, order, family, genus, and species levels are shown successively from inside to outside. Solid nodes represent the microbial groups that play important roles in SCNE, SCNM, SCPE, SCPM, SDNE, SDNM, SDPE, and SDPM. SCNE: Sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity.

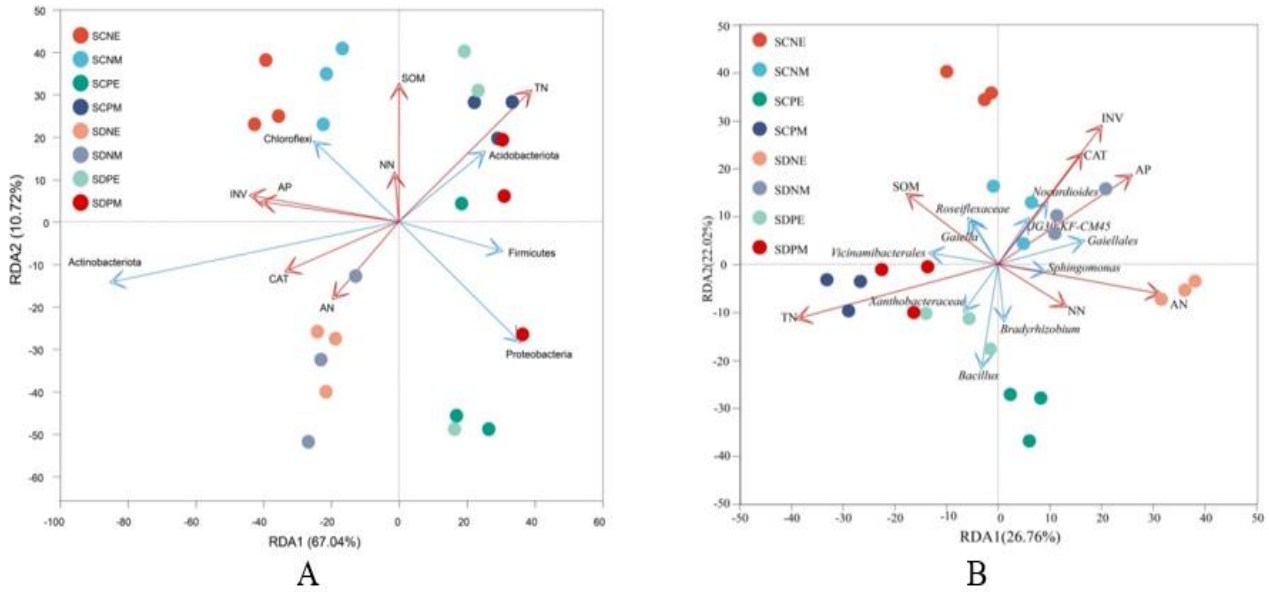

Figure 4. Redundancy analysis (RDA) at the phylum level (A) and the genus level (B). SCNE: Sugarcane monoculture new plant elongation; SCNM: sugarcane monoculture new plant maturity; SCPE: sugarcane monoculture perennial root elongation; SCPM: sugarcane monoculture perennial root maturity; SDNE: sugarcane-duck symbiosis new plant elongation; SDNM: sugarcane-duck symbiosis new plant maturity; SDPE: sugarcane-duck symbiosis perennial root elongation; SDPM: sugarcane-duck symbiosis perennial root maturity.

DISCUSSION

Soil is the main carrier of crop growth, and its nutrient level directly affects the growth and development of crops at all stages. The combination of planting and breeding also affects the physicochemical properties of the soil (Pontes et al., 2021). In this study, the growth stages of sugarcane were selected for measurement since the elongation period represents the stage when the sugarcane plant grows the most vigorously, while maturity is the period when the soil condition is more stable. Soil pH is an indicator of the soil environment and primarily affects the stability of the soil environment (Zhao et al., 2014). This study showed that SDS cultivation caused the soil pH to fluctuate in the range of 5.6-7.0, effectively maintaining the balance of soil acidity and alkalinity and creating a favorable environment for sugarcane growth and development as well as soil microbial activities, which were consistent with those of Shen et al. (2013). Two years of SDS resulted in a better-maintained sugarcane soil with enhanced fertility. The SOM and NN showed a significant increase, which may be owing to the influence of duck feed and droppings and other inputs into the soil, effectively replenishing and improving the soil. In addition, meat duck forage for weeds, which reduces the weeds' uptake of available N. Long-term disturbance accelerated the soil mineralization capacity, which is conducive to the accumulation of soil available N. This was consistent with the results of previous studies (Frei and Becker, 2010). We found that long-term SDS resulted in soil P deficiency. This slightly differed from the results of previous studies, owing to growth and nutrient uptake by sugarcane plants and the fixation of compounds such as Fe and Al oxides (Chen et al., 2012), or accelerated P release from duck activities.

Soil enzyme activity is an important indicator of soil fertility and function and is widely involved in the cycling and metabolic activities of soil C, N, and P (Zi et al., 2018). The present study showed that CAT and URE activities were significantly lower in year-two SDS compared to SC. The trampling by ducks in SDS increased the soil compactness and soil bulk density, thereby changing the composition of the microbial

communities, which affects soil enzyme activities. This is in contrast to the findings of Pretty (2008), suggesting that the lack of moisture in sugarcane field planting may affect soil enzyme activities. Low soil pH also inhibits soil URE and CAT activities (Zhao et al., 2014), which in turn affects the productivity of sugarcane and its yield quality.

Changes in crop cultivation practices can affect the soil bacterial diversity and community structure in complex farmland ecosystems (Nie et al., 2018). The present study showed that the rhizobacterial community diversity and richness of SDS were slightly poorer than those of SC during the same period. However, at the SDPE stage, the Shannon-, ACE-, and Chao1 indices were significantly improved compared with those at the SCPE stage. The reason may be that the SOM and soil nutrients in the year-one SDS soil had not yet shown a cumulative effect, which would affect the growth of the bacterial community, whereas the highly significant decrease in pH directly affected the activity of microorganisms (Zhao et al., 2014). In the year-two SDS, SOM and nutrients had significantly improved. However, the soil was tight and lacked air permeability, which in turn affected the colonization and growth of soil bacteria owing to the field activities of meat ducks and lack of mid-range ploughing and loosening agronomical measures (Huang et al., 2022). The microecosystems in the year-two SDS were more complex than those in the year-one SDS fields, with higher population diversity and richness indices. This may be because long-term inputs such as excreta and residual feed from meat ducks promote the accumulation of SOM, TN, NN, and AN in the soil (Osman et al., 2019), which creates conditions for the rapid growth of soil bacteria and simultaneously encourages exogenous bacteria to enrich bacterial diversity.

The number of OTUs at the elongation stage was lower than that at the maturity stage for all 24 soil samples from the different treatment groups. A possible reason was that the nutrient demand of sugarcane in the elongation period was large and most of the nutrients in the rhizobacteria were rapidly transferred to the above-ground sugarcane, causing the soil microorganisms to be in a competitive disadvantageous position. In contrast, the maturity period is at the end of the sugarcane growth period, the nutrient demand is relatively small, and the above- and below-ground sugarcane synchronize accumulation, which is more conducive to the growth and reproduction of soil bacteria. The community composition of SDS was similar to that of SC in the perennial root extension stage. The more acidic environment deviated from the optimal pH range (6.5-7.5), and the activities of INV, URE, and CAT were very similar between SC and SDS, resulting in a convergence of the bacterial community structure.

The soil bacterial community structure is susceptible to various factors such as soil type, cropping practices, cultivated crops, soil nutrients, and soil physical structural properties (Nie et al., 2018). At the phylum level, we found Actinobacteriota, Proteobacteria, Chloroflexi, Acidobacteriota, and Firmicutes as the dominant flora, which demonstrated their superb survivability in the agroecosystems in line with the results of previous studies (Radujkovic et al., 2018; Ma et al., 2022b). Both PCoA and LEfSe analysis showed significant differences in the compositional and distributional proportions of the dominant flora in different growth stages of SC and SDS, suggesting that SDS changed the composition of the sugarcane rhizosphere soil bacterial community to a certain extent. Previous studies showed that the abundance of flora belonging to the Actinobacteria phylum gradually decreased during sugarcane growth, while the abundance of flora such as Proteobacteria and Acidobacteria (which are more favorable for N fixation) gradually increased (Zhang et al., 2020). The decrease in the relative abundance of the Actinobacteria phylum observed in SDS would contribute to the accumulation of soil SOM, whereas the rapid increase in the Acidobacteria phylum would contribute to the decomposition of humus and maintenance of soil ecosystem health (Calleja-Cervantes et al., 2015). Lyu et al. (2023) reported that the Chloroflexi phylum was closely associated with continuous cropping, which increased the number of disease-causing bacteria and inhibits the growth of above-ground plants. A higher proportion of Chloroflexi leads to a greater likelihood of crop degradation, which indirectly suggests that the abundance of the Chloroflexi phylum in the SDS model is lower than that in an SC condition and the crop growth advantage of Chloroflexi is superior to that of the SC. The gradual enrichment of Firmicutes seen in the SDS treatment can produce metabolites such as antibiotics, sugars, polyamines, and amino acids,

which promote crop osmotic regulation, further enhancing crop tolerance and resilience, mitigating biotic stress, and suppressing soil pathogens (Dubourg et al., 2013).

At the genus level, the cultivation conditions significantly affected the abundance of *norank_f__norank_o__Gaiellales*, *Sphingomonas*, *norank_f__Roseiflexaceae*, *Bacillus*, *norank_f__Xanthobacteraceae*, *norank_f__JG30-KF-CM45*, *Nocardioides*, *norank_f__norank_o__Vicinamibacterales*, *Gaiella*, and *Bradyrhizobium* in the rhizosphere soils of sugarcane, with *norank_f__norank_o__Gaiellales* and *Sphingomonas* having the highest abundance in the year-one SDS. *Norank_f__norank_o__Gaiellales* is considered a soil probiotic and is mainly involved in C and N metabolism with a N fixation function, which also has a key role in inhibiting soil root rot (Tao et al., 2020). *Sphingomonas* can decompose soil phenolics and polysaccharides with acid-producing and peroxidase enzymes. *Sphingomonas* is an oligotrophic bacterium with P-solubilizing and N-fixing functions (Hu et al., 2016). The results of LEfSe analysis showed that SDNE was significantly enriched with *norank_f__JG30-KF-AS9*, *Sphingomonas*, *Acidothermus*, and *Chujaibacter*. The abundance of *norank_f__JG30-KF-AS9* was increased (Ma et al., 2022b), which was more than SC. *Acidothermus* has a high cellulose decomposition capacity, which can increase the SOM content (Lin et al., 2022), and *Sphingomonas* belonged to the beneficial bacteria of the SDS. The SDNM was enriched with *Nocardioides*, a genus of bacteria that solubilizes P, produces Fe, and fixes N (Wang et al., 2011). The SDPE showed a significantly increased abundance of *norank_f__Xanthobacteraceae*, which is a key component in the relationship between rhizosphere metabolites and soil microorganisms (Wang et al., 2023). Furthermore, SDPE was enriched in *Bacillus*, a genus mainly involved in the decomposition of carbohydrates, improving soil fertility, ameliorating the soil, suppressing pathogenic microorganisms, and alleviating continuous cropping disorder (Rajer et al., 2017). This suggests that *Bacillus* is a beneficial bacterial group in the early stages of SDS perennial root growth. SDPM was significantly enriched in *norank_f__norank_o__Vicinamibacterales* in the phylum Acidobacteria. This suggests that SDS can slow the decline of beneficial microbial communities triggered by continuous cropping and maintain the AP content in soil.

In this study, the soil bacterial community structure was closely related to SOM, TN, NN, AN, AP, CAT, and INV. The TN content and INV activity were identified as the most important drivers of the soil bacterial community, which is in general agreement with the results of previous studies (Cao et al., 2021). Soil nutrients are an energy source for soil microorganisms but also depend on the soil microorganisms for supplementation by transformation and decomposition; therefore, there is a complex relationship between microorganisms and soil nutrients. Furthermore, the patterns of different combinations of planting and feeding treatments on the structure of the sugarcane rhizobacterial community in this study were only short-term effects and these results still need to be clarified by long-term localization experiments on specific microbial taxa and their roles in sugarcane growth promotion.

CONCLUSIONS

In conclusion, this study showed that soil organic matter (SOM), total N (TN), nitrate N (NN), available N (AN), and available P (AP) contents in the soil were nutrient indicators with high contributions to the composition of soil bacterial communities. In contrast, the soil pH was more balanced throughout the experimental period and its effect on the soil bacterial community was nonsignificant. The dominant phylum Actinobacteria was highly significantly positively correlated with catalase (CAT) and invertase (INV) activities. The soil bacterial community composition was more fixed at the phylum level during the different growth stages in sugarcane-duck symbiosis (SDS) and sugarcane monoculture (SC), but the abundance of different species varied considerably between treatments and growth stages. The SDS significantly affected soil fertility, enzyme activity, and soil bacterial community structure and diversity in the sugarcane fields. The SOM, TN, NN, AN, AP, CAT, and INV were closely associated with the diversity of the soil bacterial communities, and TN and INV were the main driving factors. The SDS influences the fertilizer supply capacity and soil quality of sugarcane soils by regulating major soil nutrient content, available nutrients, and microbial diversity. This study provides a theoretical basis for the scientific management of SDS and the screening of biocontrol bacteria.

Author contribution

Conceptualization: Y-Z.H., W-Q.M. Methodology: D-J.B. Formal analysis: Q-S.W. Investigation: W-Q.M., D-J.B. Resources: L-Q.T. Data curation: Q.G. Writing-original draft: W-Q.M. Writing-review & editing: Y-Z.H. Visualization: J-L.W. Supervision: Y-Z.H. Project administration: L-Q.T. Funding acquisition: W-Q.M. All co-authors reviewed the final version and approved the manuscript before submission.

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