agronomy

MDPI

Article

Can Sugarcane Yield and Health Be Altered with Fully Mechanized Management?

Jian Xiao [1,2,3], Tian Liang [2], Shangdong Yang [1,2,*] and Hongwei Tan [2,*]

[1] Guangxi Key Laboratory of Agro-Environment and Agro-Products Safety, National Demonstration Center for Experimental Plant Science Education, Agricultural College, Guangxi University, Nanning 530004, China
[2] Guangxi Key Laboratory of Sugarcane Genetic Improvement, Guangxi Academy of Agricultural Sciences, Nanning 530007, China
[3] Longping Branch, College of Biology, Hunan University, Changsha 410125, China
* Correspondence: ysd706@gxu.edu.cn (S.Y.); hongwei_tan@163.com (H.T.)

Abstract: At present, fully mechanized cultivation (FMC) has begun to be utilized in commercial sugarcane production in China. To provide new insights into whether cane yield and health are altered by fully mechanized cultivations, the cane yield and endophytic microbial community structure in stems of sugarcane that underwent fully mechanized cultivation (FMC) and conventional artificial cultivation (CAC) were compared. The results showed that the diversity and richness of endophytic microorganisms, except for the bacterial richness in the stems of sugarcane, could be significantly increased by using FMC. Meanwhile, in comparison with CAC, the relative abundance of Proteobacteria and Ascomycota increased under FMC. Moreover, some dominant endophytic bacterial genera, such as *Acidovorax*, *Microbacterium*, and *Paenibacillus*, and some dominant endophytic fungal genera, such as *Scleroramularia*, *Tetraplosphaeria*, and *Dinemasporium*, were found to be significantly enriched in cane stems under FMC treatments. Additionally, the endophytic microbial functions in sugarcane stems were not significantly altered by FMC treatments. Our results suggest that cane growth, yield, and health are not significantly altered by FMC. The results also indicate that fully mechanized management can be developed as a sustainable method in sugarcane production.

Keywords: sugarcane; fully mechanized cultivation; endophytic microbial community structure

check for updates

Citation: Xiao, J.; Liang, T.; Yang, S.; Tan, H. Can Sugarcane Yield and Health Be Altered with Fully Mechanized Management? *Agronomy* **2023**, *13*, 153. https://doi.org/10.3390/agronomy13010153

Academic Editors: Yong-Xin Liu and Peng Yu

Received: 24 November 2022
Revised: 29 December 2022
Accepted: 30 December 2022
Published: 3 January 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Sugarcane (*Saccharum officinarum* L.), an important economic tropical crop cultivated worldwide, provides 80% of the world's sugar production and is also a crucial source of biofuel for ethanol production [1–3]. China is the third-most prominent sugar-producing country in the world. Recently, 75% and 90% of total sugar production, globally and in China, respectively, came from sugarcane [4]. In China, approximately 90% of the sugarcane crops are planted in the southern and southwestern regions, including Guangxi, Guangdong, and Yunnan provinces. In particular, Guangxi Province is the top sugarcane production area, accounting for more than 65% of sugar production in China since 1993 [5]. However, the steadily rising cost of labor in sugarcane cultivation has greatly increased the costs of the sugar industry. Savings in time, energy, and costs are advantages of agricultural mechanization [6]. Moreover, the productivity of agricultural land and processing efficiency can also be substantially increased by mechanization [6]. To develop a sustainable sugarcane industry, upgrading the agricultural mechanization and equipment used for sugarcane production is necessary [7,8].

Plant endophytic microbiomes have been shown to occur in both the cooperative and competitive interactions of plants. Although the cooperating endophytic microbiome performs beneficial functions for the plant, the competing microbiome has negative consequences [9]. Endophytic bacteria often display well-controlled multiplication inside plant niches, which is modulated by the plant's defense system [9,10]. The effects of the

endophytic microorganisms on the host can be described as alleviating the host's abiotic stress; protecting the host against biotic stress (pathogens and herbivores); and providing nutritional support to the host by boosting nitrogen, phosphate, iron, and other nutrients [11]. Endophytes have been isolated from a variety of plant species, with the majority of them occurring in the host plant's rhizosphere, where they enter host plants through the roots and colonize the roots' intercellular spaces [12].

However, previous studies on the fully mechanized management of sugarcane have mainly explored its effects on soil (health and fertility) and sugarcane yield. Endophytic microbial communities have rarely been used for evaluation. In particular, the collective response of endophytic microbial (bacterial and fungal) systems in stems of sugarcane, including the microbial community structures, functions, and symbiotic network patterns, to the successive mechanized management of sugarcane fields has not been reported.

The aim of this study is to answer the following questions: (1) Can the endophytic microbial communities in the stems of sugarcane be altered by fully mechanized management? (2) What kinds of endophytic microorganisms are specially or significantly enriched in the stems of sugarcane under fully mechanized management? (3) How does fully mechanized cultivation affect the symbiotic network patterns of endophytic bacteria and endophytic fungi? We anticipate that our findings will provide new insights into the effects of mechanized cultivation on modern agricultural production.

2. Materials and Methods

2.1. Study Site

The experiment was conducted at the Experimental Base of the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, which is located in Longan County (107°598″ E and 23°637″ N), Guangxi, China. The experimental site is located in the subtropical monsoon climate zone, which is rich in sunshine and rainfall. The mean summer and winter temperatures are 32 °C and 24 °C, respectively. The average annual temperature is approximately 21.7 °C, and the annual precipitation is approximately 1227–1691 mm, with rainfall mostly concentrated from June to September. The soil type is dominated by Quaternary red soil.

2.2. Experimental Design and Implementation

Firstly, the experiment was carried out beginning in the spring of 2019. The sugarcane cultivar Guitang 44 was used in this study. Two treatments were implemented as follows: (1) the fully mechanized cultivation of sugarcane (FMC, i.e., land preparation, sowing, and harvesting are all carried out by using different machines), and (2) the conventional artificial cultivation of sugarcane (CAC, i.e., land preparation, sowing, and harvesting are all carried out by hand labor operations only) as a control. Meanwhile, there were three replicate plots per treatment, and the size of each plot was 667 m^2.

The processes of FMC are described as follows: (1) For land preparations, weeding and deep plowing (mean depth of plowing is about 40 cm) were carried out first using tillage machinery (1LHT-440, Kaifeng, China); second, the rocks were cleaned up, and the roots or leaves of the sugarcane were broken up using a disc harrow (1GKN-300, Lianyungang, China); and third, furrowing was conducted using a furrowing machine (1LK-3D, Nanning, China). (2) Fertilizing, sowing, and mulching were performed simultaneously using a combined planter (2CZY-2, Beijing, China). (3) Land leveling was conducted after fertilizing, sowing, and mulching by using land-leveling equipment (3ZPF-1.36, Nanning, China). (4) During sugarcane growth, the actions of cultivation, fertilization, and banking were conducted several times by using a cultivator-hiller (3ZFS-2, Xuzhou, China). (5) Cane harvesting was conducted using a cane harvester (Austoft-4000, London, OH, USA).

The same treatments were performed identically for the CAC of sugarcane. However, all the above processes were performed by hand. The sowing density was approximately 90,000 buds per ha. All plots were fertilized with 300 kg ha^{-2} of urea, 75 kg ha^{-2} of K_2O, and 300 kg ha^{-2} of calcium superphosphate per season [4]. At the seedling and

elongation stages of sugarcane, top dressings with 30% and 70% of the total fertilizer usage, respectively, were applied.

2.3. Plant Sampling

Plant samples were collected after a 3-year experimental setup in the early harvesting stage (December 2021), and six plant samples were obtained from each plot using a S-sampling technique and mixed as biological replicates. Each treatment was replicated three times. Plant samples were collected randomly according to the method described by Yang et al. [5] and Xiao et al. [13]. First, these samples were placed in sealed sterile bags and labeled for return to the lab. Second, the stem samples were rinsed and wiped for 2 min with sterile water by using a soft brush to remove impurities from the cane surfaces, and then were washed with 75% ethanol for 1 min, following a wash with a 1% NaClO solution for 3 min. Finally, all the stems were washed with sterile water for 0.5 min, and then sterile paper was used to remove surface water [14]. To determine the success of the sterilization of the cane surface, 100 µL of water from each washing step was placed on a Luria–Bertani (LB) agar plate (g/L) (NaCl-10, tryptone-5, yeast extract-5, and agar-20) and incubated at 25 °C for 7 d. No colonies developed on the plates, confirming that they were thoroughly sterilized. The sterilization of the stem surface samples was completed before detection and analysis of the endophytic microorganisms [15]. The stems were placed in sterile bags and stored at −80 °C for pending DNA extraction.

2.4. Analysis of Microbial Diversity

Microbial community genomic DNA was extracted from stem samples using a E.Z.N.A.® DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. The DNA extract was analyzed on a 1% agarose gel, and the DNA's concentration and purity was determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA). PCR amplification and sequencing of the total DNA extracted from the plant samples were performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China. The endophytic bacterial primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1192R (5′-ACGGGCGGTGTGTRC-3′) from the V5-V7 region (endophytic bacterial 16S rRNA gene) were amplified first, and the primers 799F (5′-AACMG GATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCTTCC-3′) from the V5-V7 region were amplified second; meanwhile, ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTC ATCGATGC-3′) primers were employed to amplify the fungal ITS1 region via a ABI GeneAmp® 9700 PCR thermocycler (ABI, Vernon, CA, USA) using standard PCR protocols and conditions. The PCR products were recovered using 2% agar-gel electrophoresis, purified using an AxyPrep DNA Gel Extraction Kit (Axygen, New York, NY, USA), and quantified using a Quantus fluorometer (Promega, Madison, WI, USA). The purified amplicons were pooled in equimolar quantities and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (accession number: SRP371574).

2.5. Statistical Analyses

The experimental data were analyzed using Excel 2019 and SPSS Statistics 21.0 (IBM Corp., Armonk, New York, NY, USA). A T-test and Wilcoxon rank-sum test were used to analyze the significant differences in the statistical analyses ($p < 0.05$).

Quantitative insights into microbial ecology (QIIME) (version 1.17) was used to truncate the 300 bp reads (average quality score <20 over a 50 bp sliding window). Operational taxonomic units (OTUs) with a 97% similarity cut-off were clustered using UPARSE (version 7.1, http://drive5.com/uparse/, accessed on 9 April 2022), and chimeric sequences were identified and removed [16]. The taxonomy of each OTU representative sequence was analyzed via the RDP Classifier (http://rdp.cme.msu.edu/, accessed on 9 April 2022) against the 16S and ITS rRNA databases, using a confidence threshold of 0.7 [5].

Alpha diversities of the bacterial and fungal communities were calculated using Mothur (version v.1.30.2, https://mothur.org/wiki/calculators/, accessed on 9 April 2022). The Shannon and Ace indices were used to represent the diversity and richness of the endophytic microbial (bacterial and fungal) community, respectively. Meanwhile, a Wilcoxon rank-sum test was also performed to evaluate the diversity and richness of the microbial communities under the FMC and CAC treatments ($p < 0.05$). A principal component analysis (PCA) based on the unweighted UniFrac and a partial least squares discriminant analysis (PLS-DA) was performed to evaluate the extent of the similarity of the endophytic microbial communities, and the R language (version 3.3.1) tool was used for statistical analysis and graphing [13]. OTU tables with a 97% similarity level were selected for microbial community composition and Venn diagram analysis, and the R language (version 3.3.1) tool was used for statistics and graphing. The vegan package of the R language (version 3.3.1) tool was used and graphed for microbial community heatmap analysis. A linear discriminant analysis (LDA) was performed using LEfSe (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload, accessed on 9 April 2022) on samples according to different grouping conditions that were based on taxonomic composition to identify clusters that had a significant differential impact on sample delineation [3]. A correlation network analysis was performed by using NetworkX on the plant samples. BugBase was used for the phenotypic prediction of the microbiome. BugBase (https://bugbase.cs.umn.edu/index.html, accessed on 9 April 2022) was used to identify the high-level phenotypes present in microbiome samples and enabled the use of phenotype prediction as a microbiome analysis tool. PICRUSt was used to estimate the functional components of bacterial communities using the Kyoto Encyclopedia of Genes and Genomes (KEGG) dataset [13]. Functional predictions of the fungal communities were performed with the Fungi Functional Guild (FUN Guild) tool [16]. An online data analysis was conducted using the free online platform Majorbio Cloud Platform (www.majorbio.com) from the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The data were visualized by ImageGP (https://onlinelibrary.wiley.com/doi/10.1002/imt2.5, accessed on 9 April 2022).

3. Results

3.1. Sugarcane Yields

In comparison with the CAC treatment, the cane yields increased by 13.84%, 16.88%, and 15.57% under the FMC treatment in 2019, 2020, and 2021, respectively (Table 1). The difference between the FMC and CAC treatments was not statistically significant ($p > 0.05$). However, the results still indicate that the cane yields could be improved by the FMC treatment.

Table 1. Cane yields between FMC and CAC treatments (t ha^{-1}).

Treatments	2019	2020	2021
FMC	100.25 ± 2.01 a	98.58 ± 0.89 a	101.94 ± 0.80 a
CAC	88.06 ± 0.64 a	84.34 ± 0.67 a	88.21 ± 0.71 a

All data are presented as the mean ± standard deviation (SD). A T-test was performed ($p < 0.05$). Same letters within a column indicate no significant differences among treatments at $p > 0.05$. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

3.2. Diversity of Endophytic Bacteria and Fungi in Sugarcane Stems

The results show that the endophytic microbial diversity (Shannon) and richness (Ace) indices of the sugarcane stems under the FMC treatment were not significantly different from stems under the CAC treatment (Figure 1a–d). The results suggest that the diversity and richness of the endophytic microorganisms in sugarcane stems were not significantly changed by the FMC treatment as compared with the CAC treatment.

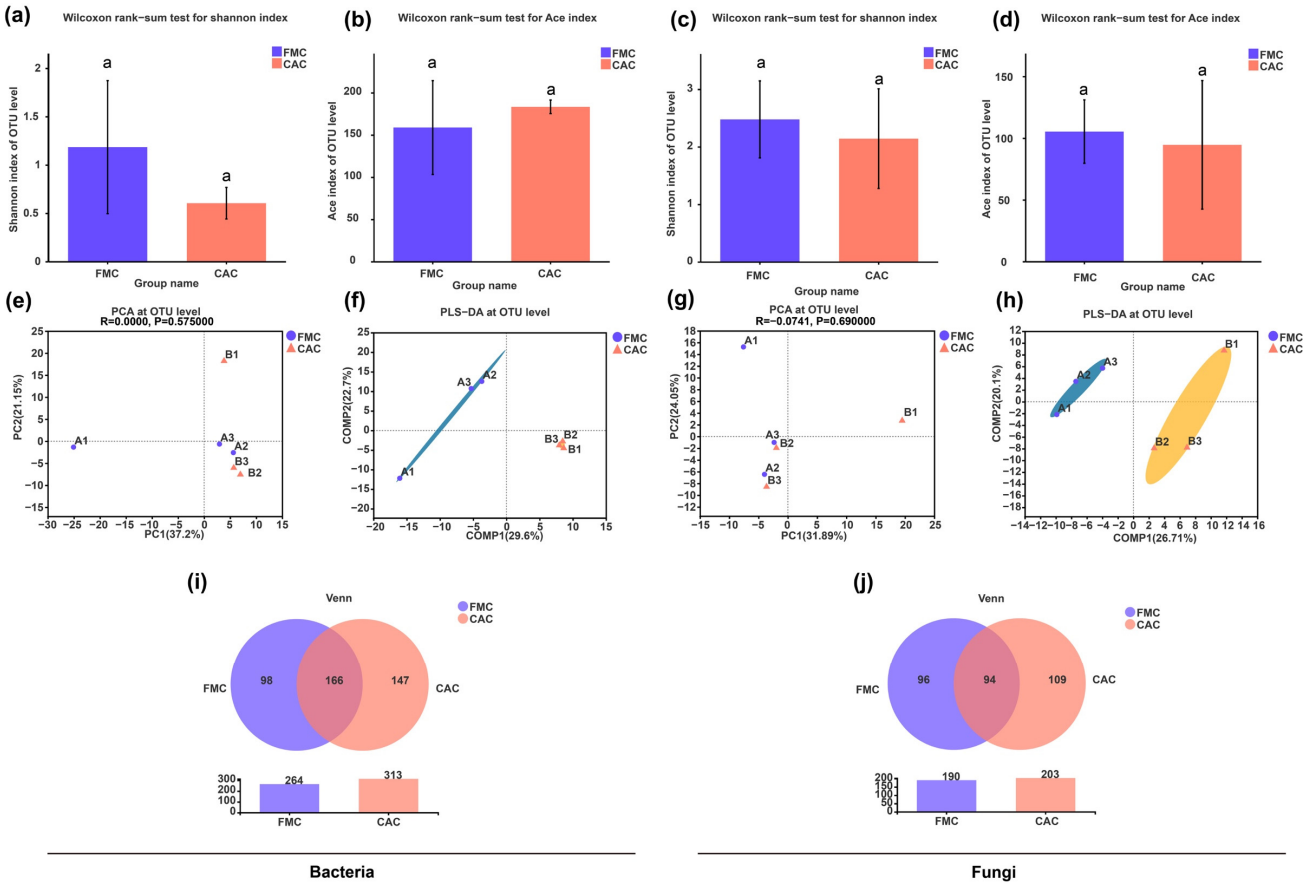

Figure 1. Comparison of endophytic microbiota structures in stems of sugarcane at a similarity level of 97% between FMC and CAC treatments (OTU level). (**a**) The Shannon index indicates endophytic bacterial diversity. (**b**) The Ace index indicates endophytic bacterial richness. (**c**) The Shannon index indicates endophytic fungal diversity. (**d**) The Ace index indicates endophytic fungal richness. (**e**) PCA of endophytic bacteria communities. (**f**) PLS-DA score plot of endophytic bacteria communities. (**g**) PCA of endophytic fungi communities. (**h**) PLS-DA score plot of endophytic fungi communities. (**i**) Venn diagram analyses of endophytic bacteria. (**j**) Venn diagram analyses of endophytic fungi. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation. Same letters on bars within a figure indicate no significant differences in mean ranks among treatments at $p > 0.05$.

The unweighted UniFrac principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were also performed to evaluate the extent of the similarity of the endophytic microbial communities at the operational taxonomic unit (OTU) level. The results showed that the microbial communities of FMC and CAC were clustered separately, but there were also similarities in the microbial compositions between FMC and CAC treatments (Figure 1e–h). In addition, both the total and the unique numbers of microorganisms in sugarcane stems under the FMC treatment were lower than those under the CAC treatment at the OTU level (Figure 1i,j).

3.3. Composition of Endophytic Bacteria and Fungi in Sugarcane Stems at Different Levels

The dominant endophytic microorganisms (bacteria and fungi) were referred to as those with relative abundance percentages greater than 1% (Figures 2 and 3). A Wilcoxon rank-sum test was also performed for the endophytic microorganisms with relative abundance percentages at a phylum and genus level ($p < 0.05$). However, the results showed that there were no significant differences in this measure between the FMC and CAC treatments.

At the phylum level, the proportions of dominant endophytic bacterial phyla in sugarcane under the CAC treatment, from high to low, were Proteobacteria at 94.11%,

Actinobacteriota at 4.81%, and others at 1.09%. In contrast, the proportions of dominant endophytic bacterial phyla in sugarcane under the FMC treatment were Proteobacteria at 96.49%, Actinobacteriota at 2.56%, and others at 0.95%. The relative abundance of Proteobacteria increased in canes under the FMC treatment as compared with those under the CAC treatment. Meanwhile, the relative abundances of Actinobacteriota and other bacteria in sugarcane under the FMC treatment were all lower than those under the CAC treatment (Figure 2a).

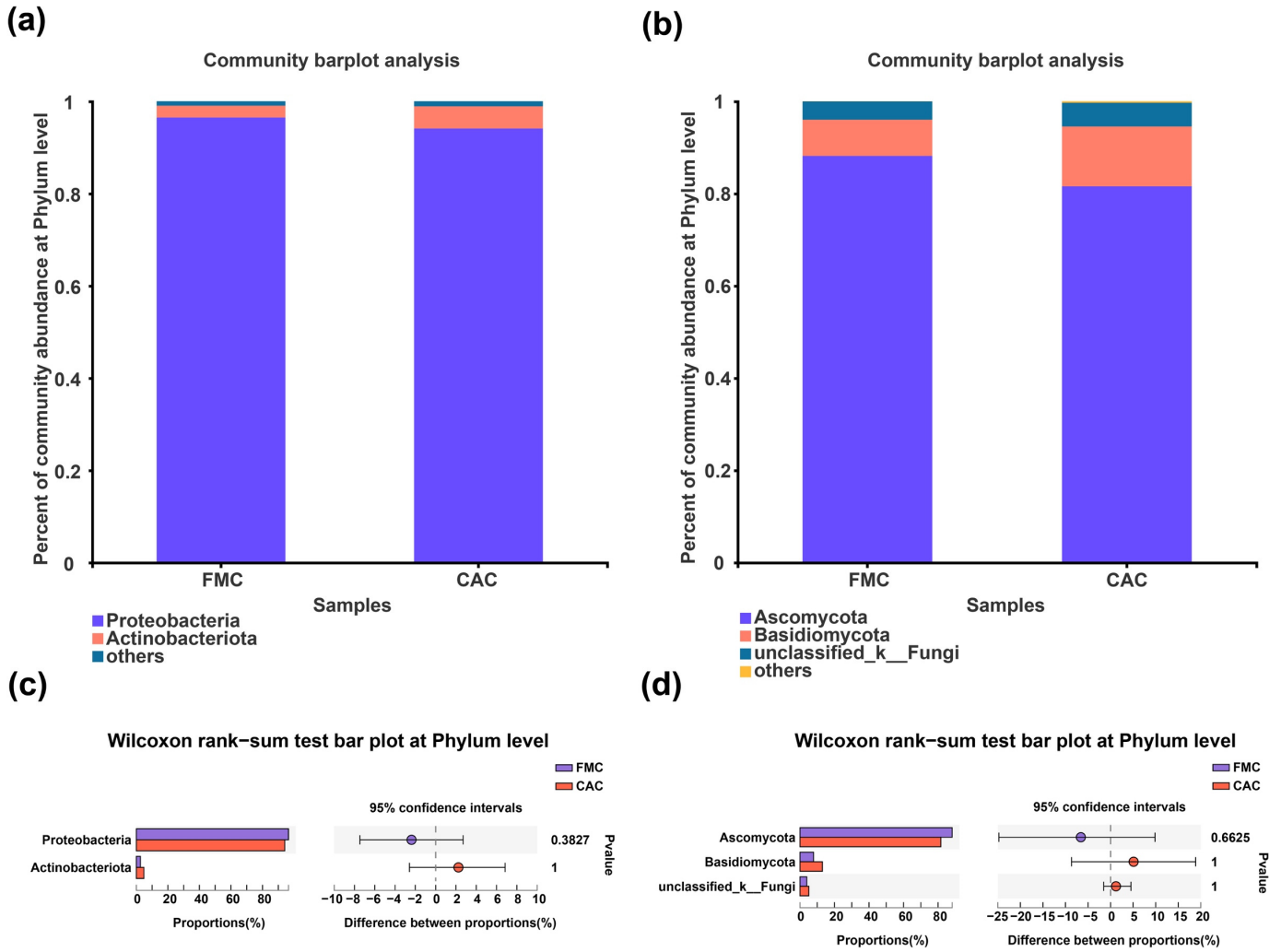

Figure 2. Compositions of endophytic microorganisms in the stems of sugarcane under FMC and CAC treatments at phylum level. (**a**) The proportions of the dominant endophytic bacteria. (**b**) The proportions of the dominant endophytic fungi. (**c**) Test for significant difference in the bacterial abundance between groups. (**d**) Test for significant difference in the fungal abundance between groups. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

In addition, the proportions of endophytic dominant fungal phyla in CAC sugarcane, from high to low, were Ascomycota at 81.56%, Basidiomycota at 12.98%, and unclassified_k__Fungi at 5.14%. By contrast, the proportions of dominant endophytic fungal phyla in FMC sugarcane were Ascomycota at 88.15%, Basidiomycota at 7.88%, and unclassified_k__Fungi at 3.95%. The relative abundance of Ascomycota increased in FMC-treated sugarcane compared with that under the CAC treatment. Meanwhile, the relative abundances of Basidiomycota and unclassified_k__Fungi were lower under the FMC treatment compared with those under the CAC treatment (Figure 2b).

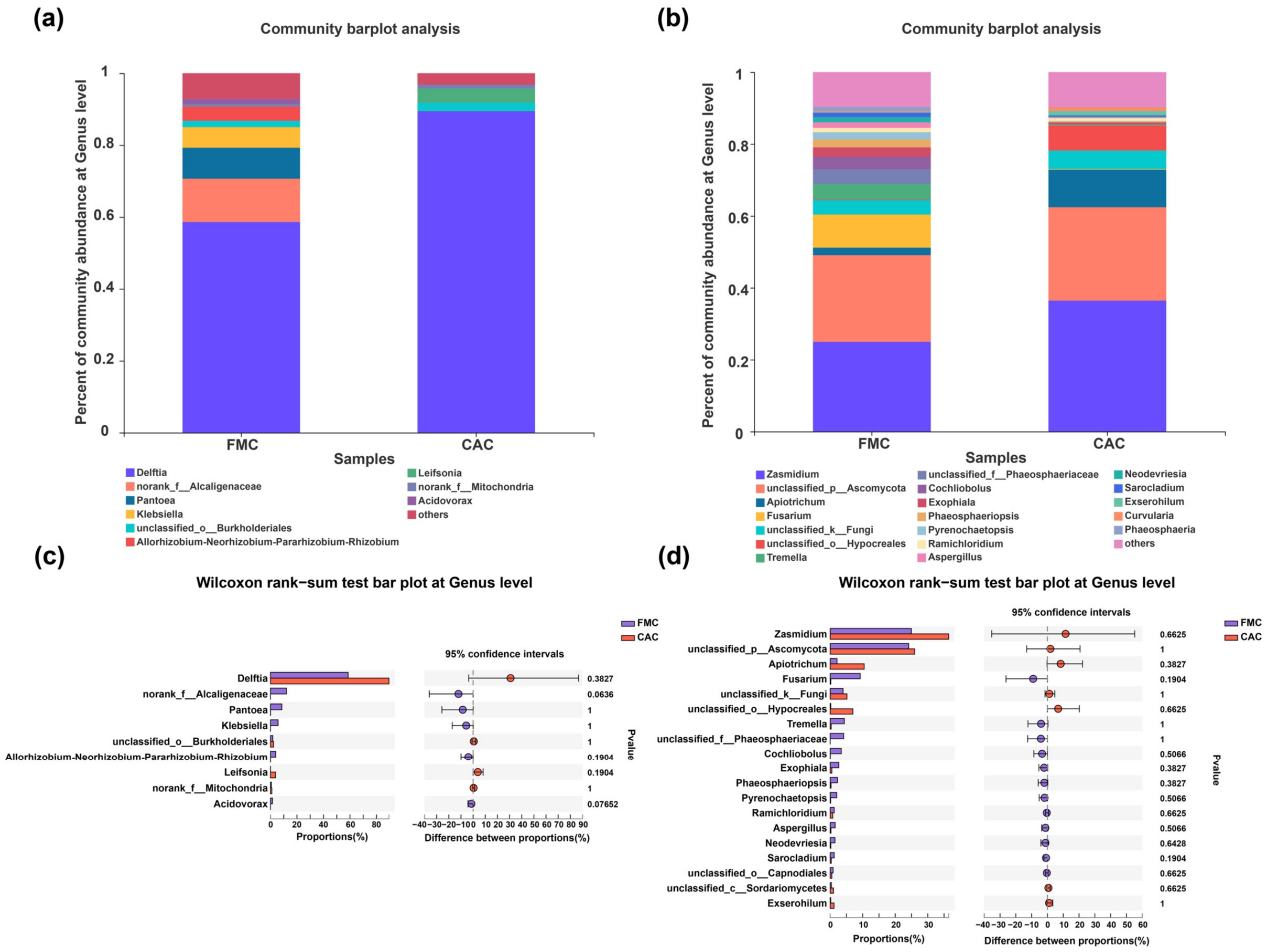

Figure 3. Compositions of endophytic microorganisms in the stems of sugarcane under FMC and CAC treatments at genus level. (**a**) The proportions of the dominant endophytic bacteria. (**b**) The proportions of the dominant endophytic fungi. (**c**) Test for significant difference in the bacterial abundance between groups. (**d**) Test for significant difference in the fungal abundance between groups. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

At the genus level, the relative abundances of *Delftia* and unclassified_o__*Burkholderiales* were lower under the FMC treatment as compared with the CAC treatment. However, norank_f__*Alcaligenaceae*, *Pantoea*, *Klebsiella*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Acidovorax* were the special, dominant endophytic bacterial genera in FMC plots. *Leifsonia* and norank_f__*Mitochondria* were the unique, dominant endophytic bacterial genera in CAC plots (Figure 3a).

Additionally, the proportions of *Zasmidium*, unclassified_p__*Ascomycota*, *Apiotrichum*, unclassified_k__Fungi, and others were also lower under the FMC treatment as compared with the CAC treatment. Moreover, *Fusarium*, *Tremella*, unclassified_f__*Phaeosphaeriaceae*, *Cochliobolus*, *Exophiala*, *Phaeosphaeriopsis*, *Pyrenochaetopsis*, *Ramichloridium*, *Aspergillus*, *Neodevriesia*, *Sarocladium*, and *Phaeosphaeria* were the special, dominant endophytic fungal genera under the FMC treatment. In contrast, unclassified_o__*Hypocreales*, *Exserohilum*, and *Curvularia* were the only unique, dominant endophytic fungal genera under the CAC treatment (Figure 3b).

An LEfSe analysis was also conducted to identify endophytic microbes in sugarcane stems under the FMC treatment. A total of 39 bacterial and 12 fungal clades (from phylum to genus) exhibited significant differences in their cladogram structure (LDA > 2.0).

As seen in Figure 4, there was no significant enrichment of dominant endophytic bacteria and fungi detected between the FMC and CAC treatments at the phylum level.

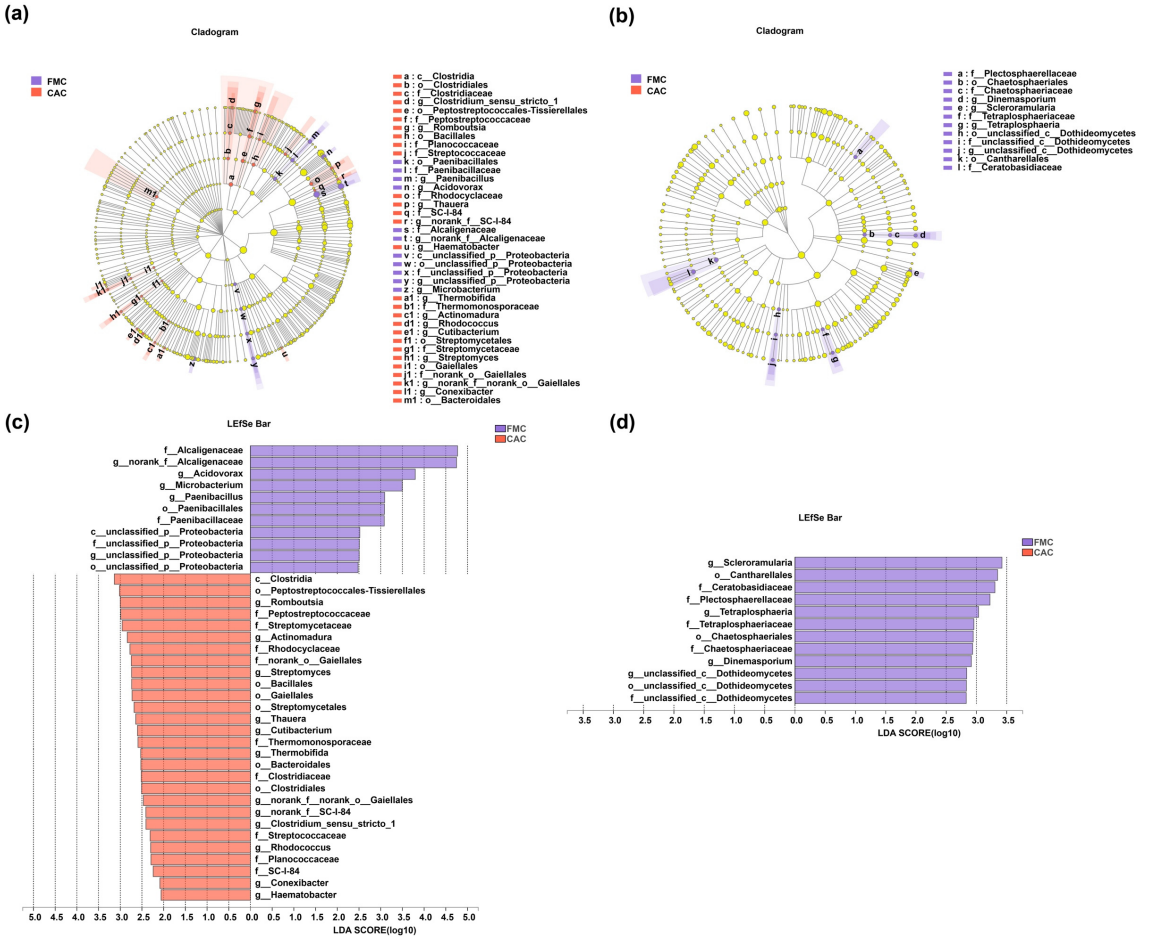

Figure 4. Cladogram showing the phylogenetic distribution of the bacterial (**a**) and fungal (**b**) lineages associated with stems of sugarcane under FMC and CAC treatments. Indicator bacteria (**c**) and fungi (**d**) with LDA scores of 2.0 or greater in microbial communities associated with stems of sugarcane under FMC and CAC treatments (LEfSe). Circles indicate phylogenetic levels from phylum to genus. The diameter of each circle is proportional to the abundance of the group. Different prefixes indicate different levels (p—phylum; c—class; o—order; f—family; g—genus). FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

Meanwhile, the endophytic bacteria, such as *Romboutsia*, *Actinomadura*, *Streptomyces*, *Thauera*, *Cutibacterium*, *Thermobifida*, norank_f__norank_o__*Gaiellales*, norank_f__SC-I-84, *Clostridium*_sensu_stricto_1, *Rhodococcus*, *Conexibacter*, and *Haematobacter*, were significantly enriched in canes under the CAC treatment at the genus level; in contrast, norank_f__*Alcaligenaceae*, *Acidovorax*, *Microbacterium*, *Paenibacillus*, and unclassified_p__*Proteobacteria* were significantly enriched under the FMC treatment at the genus level.

Moreover, in comparison with the FMC treatment, there was no significant enrichment of dominant endophytic fungi under the CAC treatment at the genus level; however, *Scleroramularia*, *Tetraplosphaeria*, *Dinemasporium*, and unclassified_c__*Dothideomycetes* were significantly enriched under the FMC treatment at the genus level.

The correlation between the significant enrichment of endophytic bacterial and fungal genera is important. Therefore, an interaction network was constructed to further elucidate the relationship between the significant enrichment of the bacterial and fungal genera. Based on the abundance of the endophytic bacterial and fungal genera, Spearman's rank correlation coefficients were calculated to reflect the correlations between them (Figure 5). The results showed that the enrichment of the endophytic fungal genera (*Scleroramularia*, *Tetraplosphaeria*, and *Dinemasporium*) was positively correlated with the enrichment of the endophytic bacterial genus *Acidovorax*.

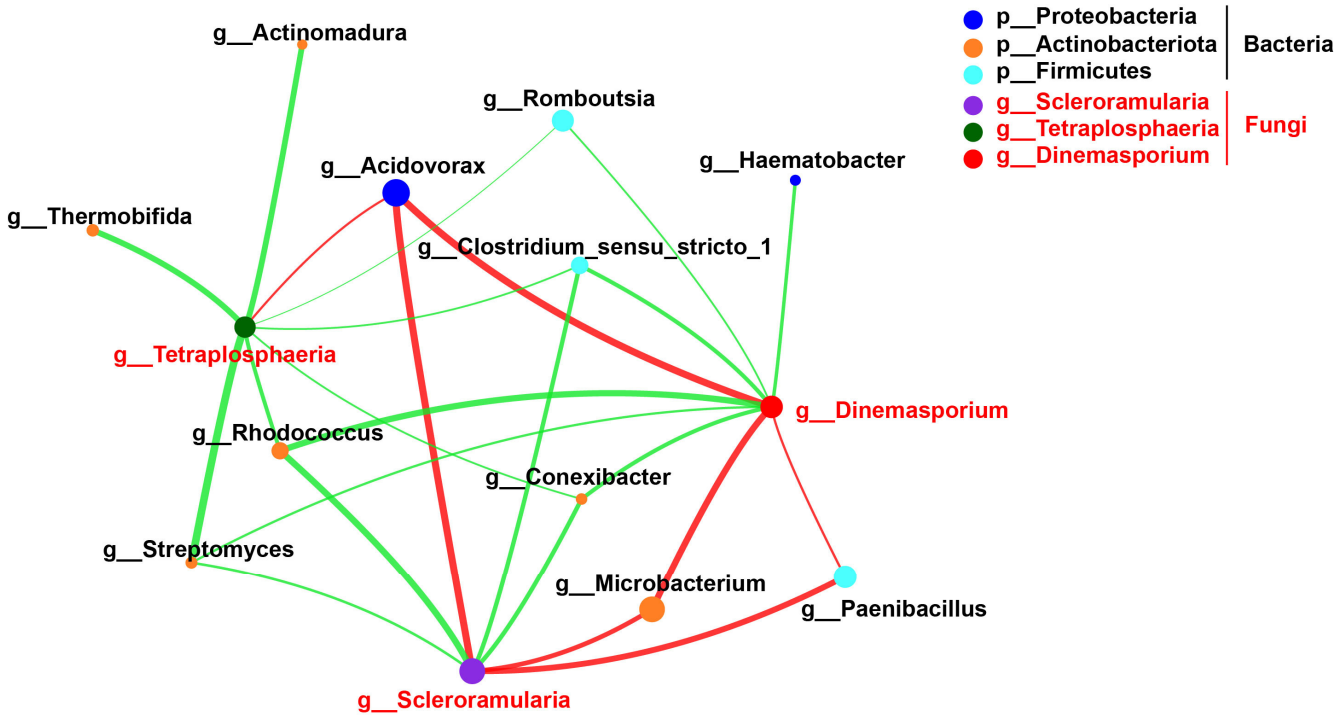

Figure 5. Correlation network analysis of significantly enriched bacterial and fungal genera. The Spearman coefficients that showed the significant enrichment of bacterial and fungal genera were also calculated to reflect the correlation between species, where the absolute value of the correlation coefficient ≥ 0.5, with $p < 0.05$. The sizes of the nodes in Figure 5 indicate the abundances of species, and different colors indicate different species; the color of the connecting lines indicates a positive and negative correlation, where red indicates a positive correlation, green indicates a negative correlation, and the thickness of the lines indicates the magnitude of the correlation coefficient. The thicker the line, the higher the correlation between species; the more lines, the closer the connection between the nodes (p—phylum; g—genus).

Based on the BugBase analysis, it was found that the endophytic bacterial phenotypes in sugarcane stems under FMC and CAC treatments were mainly classified into nine groups (Figure 6). Meanwhile, a Wilcoxon rank-sum test was also performed for the nine bacterial phenotype groups under FMC and CAC treatments ($p < 0.05$). The results showed that the abundances of these nine bacterial phenotypes were not significantly different for FMC and CAC treatments. However, the abundant percentages of Stress_Tolerant, Forms_ Biofilms, and Contains_ Mobile_ Elements in the bacterial community increased in the stems under the FMC treatment as compared with those under the CAC treatment. This result indicates that the stress resistance of sugarcane could be improved by FMC treatment.

PICRUSt2 and FUNGuild were carried out to predict bacterial and fungal functions, respectively. Meanwhile, a Wilcoxon rank-sum test was also performed to evaluate the functions of the bacterial and fungal communities under FMC and CAC treatments ($p < 0.05$). The results showed that the functions of endophytic bacteria (Figure 7a) and fungi (Figure 7b) in sugarcane under the FMC treatment were not significantly different from those under the CAC treatment.

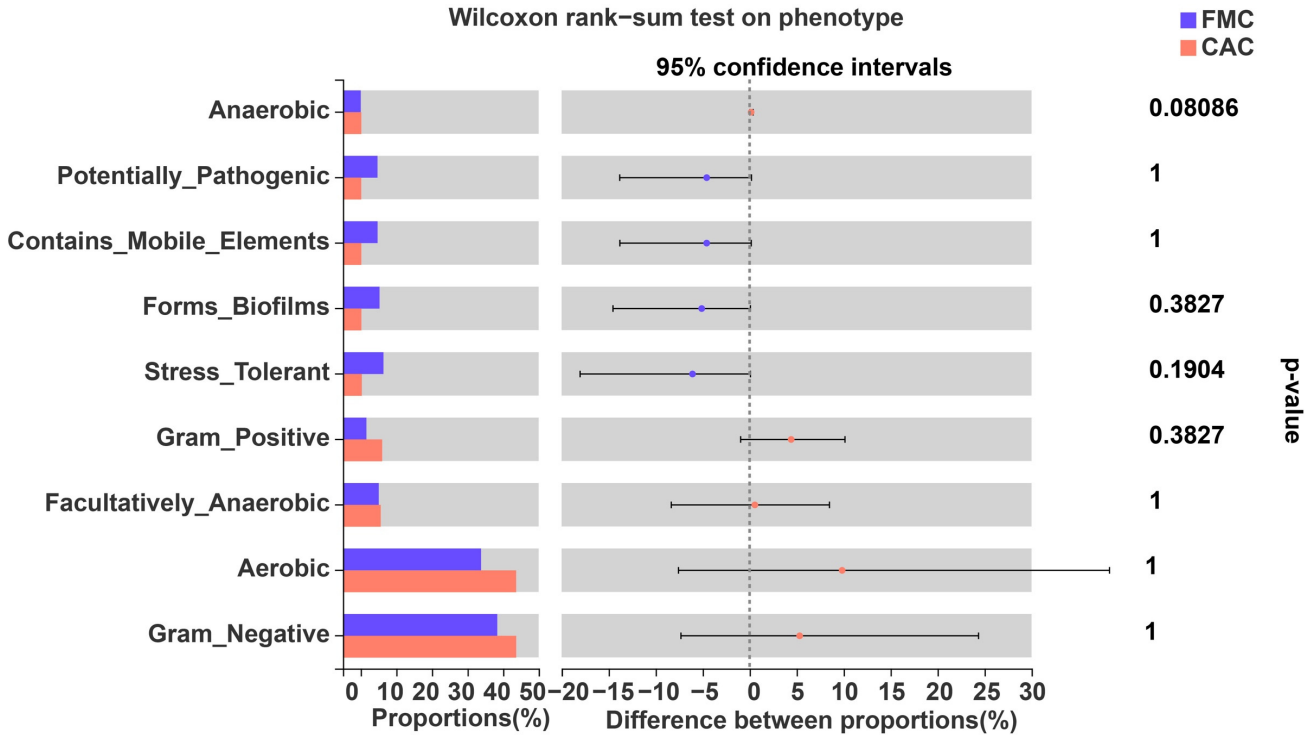

Figure 6. Endophytic bacterial community phenotypes from FMC and CAC treatments identified by BugBase-predicted analysis. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

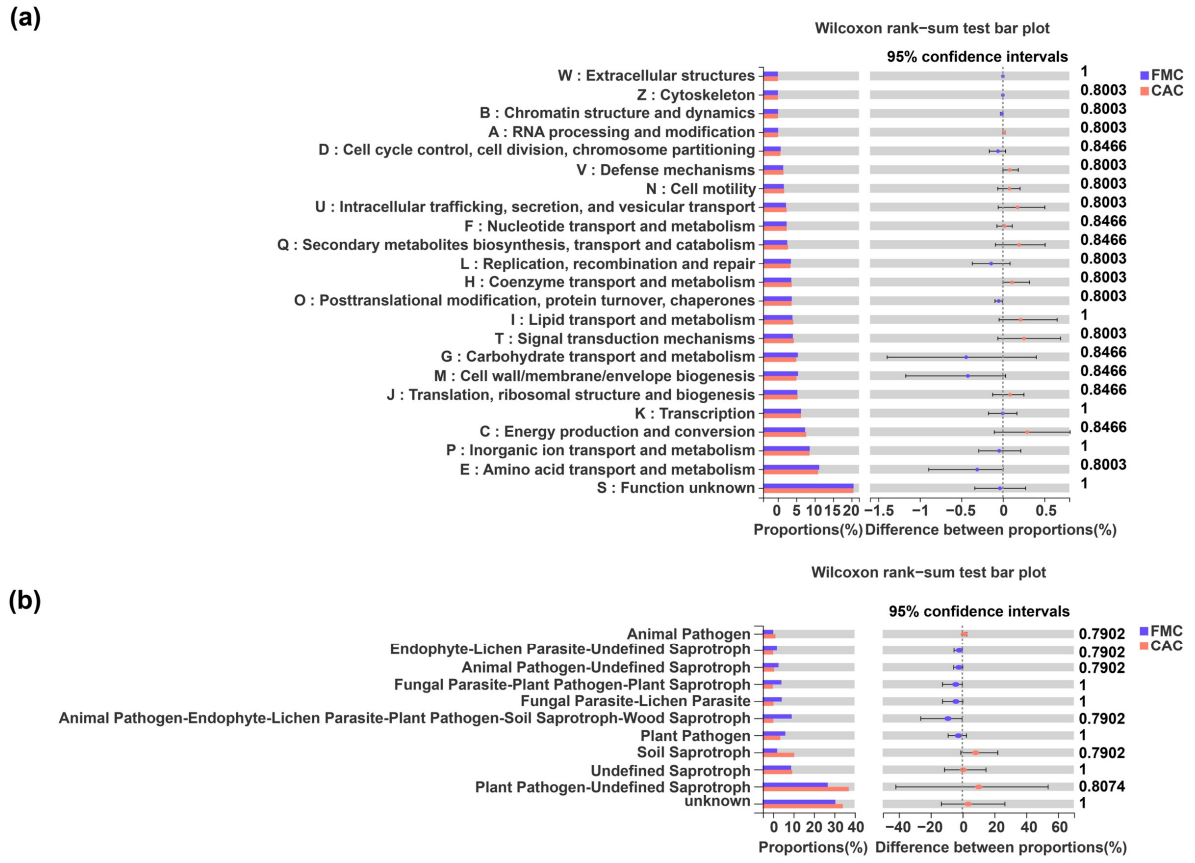

Figure 7. Functional predictions of the bacterial (**a**) and fungal (**b**) communities in stems of sugarcanes under FMC and CAC treatments. FMC—fully mechanized cultivation; CAC—conventional artificial cultivation.

4. Discussion

Hartman et al. [17] found that management type and tillage intensity were the main causes of bacteria and fungi in roots. Similarly, in comparison with the CAC treatment, we found that for the endophytic microbial compositions, the endophytic bacteria as well as the endophytic fungal compositions in sugarcane were significantly changed by the FMC treatment. For example, in comparison with the CAC treatment, the relative abundance of Proteobacteria in stems under the FMC treatment was increased. Endophytic Proteobacteria have been demonstrated to be a Plant Growth-Promoting Rhizobacteria (PGPR) [18].

At the genus level, *Pantoea*, *Klebsiella*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Acidovorax*, *Microbacterium*, and *Paenibacillus* were the special or significantly enriched dominant endophytic bacterial genera under the FMC treatment. Previous studies reported that *Pantoea* is a type of polysaccharide-producing, IAA-producing, iron carrier-producing, phosphate-solubilizing, and antagonistic-to-pathogenic fungi, and is part of the functional fungal genus. *Pantoea* also has the function of promoting plant growth and development [19,20]. Meanwhile, *Klebsiella*, as one of the nitrogen-fixing bacteria in sugarcane, can have a pro-growth effect [21]. Bigott et al. [22] also suggested that *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* might be helpful in promoting plant growth and improving resistance against abiotic stress. Moreover, *Acidovorax*, known as one of the commensal species or plant-helpful bacteria, can produce secondary metabolites and hormones to promote plant development while simultaneously being antagonistic to plant pathogens [23,24]. Furthermore, *Microbacterium*, a producer of secondary metabolites that belongs to the Gram-positive group of bacteria and is not acid-fast, can also produce carotenoids with antioxidant and coloring properties [25]. *Paenibacillus*, characterized by nitrogen fixation, phosphate solubilization, phytohormone indole-3-acetic acid (IAA) synthesis, and siderophore release, can improve crop growth. The enrichment of the dominant endophytic bacterial genera under the FMC treatment not only can help to defend against insect herbivores such as nematodes but can also resist plant pathogens [26].

Additionally, the enrichment of endophytic fungi, such as *Scleroramularia*, *Tetraplosphaeria*, and *Dinemasporium*, was also found under the FMC treatment. *Scleroramularia*, a new, potentially species-rich genus of epiphytic fungus, is often found on the fruit surfaces of several hosts. This implies that it may have many untapped niches to be investigated [27]. Meanwhile, *Dinemasporium* can produce bioactive metabolites with antibacterial, antifungal, and antialgal activities [28].

5. Conclusions

In comparison with the CAC treatment, even though the diversity and richness of endophytic microorganisms in sugarcane stems under the FMC treatment were not significantly different, the relative abundances of Proteobacteria and Ascomycota increased under the FMC treatment. Additionally, some dominant endophytic bacterial genera, such as *Acidovorax*, *Microbacterium*, and *Paenibacillus*, and some dominant endophytic fungal genera, such as *Scleroramularia*, *Tetraplosphaeria*, and *Dinemasporium*, which belong to beneficial microbes, were all significantly enriched under the FMC treatments. All of our results suggest that cane growth and health are not only positively impacted by the FMC treatment, but that this treatment could be considered a sustainable method for future sugarcane production.

Author Contributions: Conceptualization, S.Y. and H.T.; data curation, J.X.; formal analysis, S.Y.; funding acquisition, H.T.; investigation, T.L.; methodology, J.X. and T.L.; project administration, J.X. and T.L.; resources, T.L.; software, J.X.; visualization, J.X.; writing—original draft, J.X.; writing—review and editing, J.X. and S.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key R&D Program of China (2020YFD1000600), the FAO project (FAO/CPR/3804), and the Guangxi Major Science and Technology Project (GuikeAA22117006).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (accession number: SRP371574).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Singh, R.K.; Singh, P.; Li, H.B.; Song, Q.Q.; Guo, D.J.; Solanki, M.K.; Verma, K.K.; Malviya, M.K.; Song, X.P.; Lakshmanan, P.; et al. Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: A comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. *BMC Plant Biol.* **2020**, *20*, 220. [CrossRef] [PubMed]
2. Pang, Z.; Dong, F.; Liu, Q.; Lin, W.; Hu, C.; Yuan, Z. Soil metagenomics reveals effects of continuous sugarcane cropping on the structure and functional pathway of rhizospheric microbial community. *Front. Microbiol.* **2021**, *12*, 369. [CrossRef]
3. Yang, S.D.; Xiao, J.; Liang, T.; Tan, H.W. Response of bacterial compositions to the use of slow-release fertilizers with long-acting agents and synergists. *Appl. Soil Ecol.* **2023**, *182*, 104699. [CrossRef]
4. Tayyab, M.; Yang, Z.; Zhang, C.; Islam, W.; Lin, W.; Zhang, H. Sugarcane monoculture drives microbial community composition, activity and abundance of agricultural-related microorganisms. *Environ. Sci. Pollut. Res.* **2021**, *28*, 48080–48096. [CrossRef] [PubMed]
5. Yang, S.D.; Xiao, J.; Liang, T.; He, W.Z.; Tan, H.W. Response of soil biological properties and bacterial diversity to different levels of nitrogen application in sugarcane fields. *AMB Express* **2021**, *11*, 172. [CrossRef] [PubMed]
6. Fatah, G.S.A.; Verona, S.L.; Nugraheni, S.D. The analysis of labor efficiency on sugarcane cultivation through mechanization application. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *974*, 012127. [CrossRef]
7. Tayyab, M.; Fallah, N.; Zhang, C.; Pang, Z.; Islam, W.; Lin, S.; Lin, W.; Zhang, H. Sugarcane cultivar-dependent changes in assemblage of soil rhizosphere fungal communities in subtropical ecosystem. *Environ. Sci. Pollut. Res.* **2022**, *29*, 20795–20807. [CrossRef]
8. Tayyab, M.; Islam, W.; Noman, A.; Pang, Z.; Li, S.; Lin, S.; Lin, W.; Zhang, H. Sugarcane cultivars manipulate rhizosphere bacterial communities' structure and composition of agriculturally important keystone taxa. *3 Biotech* **2022**, *12*, 32. [CrossRef]
9. Kumar, M.; Kumar, A.; Sahu, K.P.; Patel, A.; Reddy, B.; Sheoran, N.; Charishma, K.; Rajashekara, H.; Bhagat, S.; Rathour, R. Deciphering core-microbiome of rice leaf endosphere: Revelation by metagenomic and microbiological analysis of aromatic and non-aromatic genotypes grown in three geographical zones. *Microbiol. Res.* **2021**, *246*, 126704. [CrossRef]
10. Ashajyothi, M.; Kumar, A.; Sheoran, N.; Ganesan, P.; Gogoi, R.; Subbaiyan, G.K.; Bhattacharya, R. Black pepper (*Piper nigrum* L.) associated endophytic Pseudomonas putida BP25 alters root phenotype and induces defense in rice (*Oryza sativa* L.) against blast disease incited by *Magnaporthe oryzae*. *Biol. Control* **2020**, *143*, 104181. [CrossRef]
11. Teheran-Sierra, L.G.; Funnicelli, M.I.G.; de Carvalho, L.A.L.; Ferro, M.I.T.; Soares, M.A.; Pinheiro, D.G. Bacterial communities associated with sugarcane under different agricultural management exhibit a diversity of plant growth-promoting traits and evidence of synergistic effect. *Microbiol. Res.* **2021**, *247*, 126729. [CrossRef] [PubMed]
12. Vandana, U.K.; Rajkumari, J.; Singha, L.P.; Satish, L.; Alavilli, H.; Sudheer, P.D.V.N.; Chauhan, S.; Ratnala, R.; Satturu, V.; Mazumder, P.B.; et al. The Endophytic Microbiome as a Hotspot of Synergistic Interactions, with Prospects of Plant Growth Promotion. *Biology* **2021**, *10*, 101. [CrossRef] [PubMed]
13. Xiao, J.; Chen, S.Y.; Sun, Y.; Yang, S.D.; He, Y. Differences of rhizospheric and endophytic bacteria are recruited by different watermelon phenotypes relating to rind colors formation. *Sci. Rep.* **2022**, *12*, 6360. [CrossRef] [PubMed]
14. Ren, F.; Dong, W.; Yan, D.H. Organs, cultivars, soil, and fruit properties affect structure of endophytic mycobiota of Pinggu peach trees. *Microorganisms* **2019**, *7*, 322. [CrossRef] [PubMed]
15. Di, Y.N.; Kui, L.; Singh, P.; Liu, L.F.; Xie, L.Y.; He, L.L.; Li, F.S. Identification and characterization of bacillus subtilis B9: A diazotrophic plant growth-promoting endophytic bacterium isolated from sugarcane root. *J. Plant Growth Regul.* **2022**. [CrossRef]
16. Xiao, J.; Chen, S.; Sun, Y.; Wu, S.; Liang, W.; Yang, S. Effects of mechanical weeding on soil fertility and microbial community structure in star anise (*Illicium verum* Hook.f.) plantations. *PLoS ONE* **2022**, *17*, e0266949. [CrossRef] [PubMed]
17. Hartman, K.; van der Heijden, M.G.A.; Wittwer, R.A.; Banerjee, S.; Walser, J.C.; Schlaeppi, K. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **2018**, *6*, 14. [CrossRef]
18. Bruto, M.; Prigent-Combaret, C.; Muller, D.; Moënne-Loccoz, Y. Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. *Sci. Rep.* **2014**, *4*, 6261. [CrossRef]
19. Ansari, F.A.; Ahmad, I. Isolation, functional characterization and efficacy of biofilm-forming rhizobacteria under abiotic stress conditions. *Antonie Van Leeuw.* **2019**, *112*, 1827–1839. [CrossRef]
20. Xiao, J.; Chen, S.Y.; Sun, Y.; Yang, S.D.; Tan, H.W. Effect of intercropping with different legume crops on endophytic bacterial diversity of sugarcanes. *Chin. J. Trop. Crops* **2021**, *42*, 3188–3198. (In Chinese) [CrossRef]
21. Mehnaz, S. Bacteria in Agrobiology: Crop Ecosystems. Plant Growth-Promoting Bacteria Associated with Sugarcane. In *Bacteria in Agrobiology: Crop Ecosystems*; Maheshwari, D.K., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 165–187.
22. Bigott, Y.; Gallego, S.; Montemurro, N.; Breuil, M.C.; Pérez, S.; Michas, A.; Martin-Laurent, F.; Schröder, P. Fate and impact of wastewater-borne micropollutants in lettuce and the root- associated bacteria. *Sci. Total Environ.* **2022**, *831*, 154674. [CrossRef] [PubMed]

23. Siani, R.; Stabl, G.; Gutjahr, C.; Schloter, M.; Radl, V. *Acidovorax* pan-genome reveals specific functional traits for plant beneficial and pathogenic plant-associations. *Microb. Genom.* **2021**, *7*, 000666. [CrossRef] [PubMed]
24. Giordano, P.R.; Chaves, A.M.; Mitkowski, N.A.; Vargas, J.M. Identification, characterization, and distribution of *Acidovorax avenae* subsp. *avenae* associated with creeping bentgrass etiolation and decline. *Plant Dis.* **2012**, *96*, 1736–1742. [CrossRef] [PubMed]
25. Xie, F.; Niu, S.; Lin, X.; Pei, S.; Jiang, L.; Tian, Y.; Zhang, G. Description of *Microbacterium luteum* sp. nov.; *Microbacterium cremeum* sp. nov.; and *Microbacterium atlanticum* sp. nov.; three novel C50 carotenoid producing bacteria. *J. Microbiol.* **2021**, *59*, 886–897. [CrossRef] [PubMed]
26. Grady, E.N.; MacDonald, J.; Liu, L.; Richman, A.; Yuan, Z.C. Current knowledge and perspectives of *Paenibacillus*: A review. *Microb. Cell Fact.* **2016**, *15*, 203. [CrossRef]
27. Li, H.; Sun, G.; Batzer, J.C.; Crous, P.W.; Groenewald, J.Z.; Karakaya, A.; Gleason, M.L. *Scleroramularia* gen. nov. associated with sooty blotch and flyspeck of apple and pawpaw from the Northern Hemisphere. *Fungal Divers.* **2011**, *46*, 53–66. [CrossRef]
28. Krohn, K.; Sohrab, M.H.; van Ree, T.; Draeger, S.; Schulz, B.; Antus, S.; Kurtán, T. Dinemasones A, B and C—New bioactive metabolites from the endophytic gungus *Dinemasporium strigosum*. *Eur. J. Org. Chem.* **2008**, *2008*, 5638–5646. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.