

Soil fungal diversity profile of the sugarcane plantations in Lampung and South Sumatra, Indonesia

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Abstract

As a high-value commodity, sugarcane harvesting areas in Indonesia have been declining year after year. Metagenomic studies on sugarcane plantation land are expected to provide an overview of land suitability for sustainable sugarcane production. This study investigated soil fungal communities in two sugarcane plantations, Bunga Mayang (BUMA) and Cinta Manis (CIMA), in Lampung and South Sumatra, respectively, using high-throughput ITS1 gene sequencing. Chemical and biological analyses were conducted on soils with depths of 0-20 cm and 20-40 cm on plant cane (PC) and ratoon cane (RC) categories. The results showed that plantation land in South Sumatra (CIMA) had higher organic carbon and total nitrogen concentrations than in Lampung (BUMA). However, in several other parameters, such as P₂O₅, K₂O, and microbial population, the BUMA farm has higher values with lower pH. Metagenomic analysis revealed the differences in microbial dominance between the plantation areas. Sugarcane fields in Lampung were dominated by the genus *Protrudomyces*, *Coralloidiomyces*, and *Spizellomyces*, while South Sumatra was dominated by the genus *Lichtheimia*, *Phyctochytrium*, and *Spizellomyces*. In the RC category, *Lichtheimia corymbifera* is the most dominant species in both the BUMA (RC-B) and CIMA (RC-C) plantations. However, in the PC category, BUMA (PC-B) is dominated by *Protrudomyces lateralis*, meanwhile CIMA (PC-C) is dominated by *Phyctochytrium californicum*. The alpha diversity values of the two areas did not significantly differ, with low *Trichoderma* populations. Thus, sugarcane fields in the two locations differ in microbial composition, which is thought to be influenced by different technical cultures and environmental conditions.

[Keywords: metagenomics analysis, soil fungal profile, sugarcane]

Introduction

Sugarcane (*Saccharum* spp.) is an important plantation commodity in several Asian countries, including Indonesia. According to Monela et al. (2022), sugarcane has significant economic value and is highly relevant to the food industry and the energy supply chain from renewable sources. Unfortunately, over the past 10 years, sugarcane productivity in Indonesia has not reached its potential, resulting in continued sugar imports (Aziz et al., 2024). Indonesia's sugarcane plantation areas are spread across 12 provinces, with the largest in East Java, Lampung, Central Java, South Sumatra, South Sulawesi, Gorontalo, and West Java. However, the sugarcane harvest area has stagnated over the past decades (2013-2022), even experiencing a slight downward trend of 0.82% per year. The limited availability of land suitable for sugarcane cultivation makes it difficult to expand sugarcane areas (Pusdatin, 2022).

Sugarcane plantations in each region have their own physical, chemical, and biological characteristics. Differences in soil characteristics affect soil health, nutrient storage capacity, and plants' ability to take up nutrients. Complex interactions between host plants and environmental conditions, including growth stages, soil compartments, and stresses, lead to significant changes in microbial diversity and function (Liu et al., 2021; Ajilogba et al., 2022). Soil is a complex ecosystem in which microbial communities play diverse roles. Plant growth-promoting bacteria (PGPB) and plant growth-promoting fungi (PGPF) can work with plants to achieve plant health and superior productivity (Malviya et al., 2021; Moneda et al., 2022). Metagenomics is a recent approach widely used to obtain a picture of microbial diversity in an object, such as soil. In addition, metagenomic studies are useful for revealing the composition, function, and network patterns of microbial communities (Liu et al., 2022).

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Metagenomic analysis in sugar cane fields is expected to provide information to assess land suitability based on soil microbial profiles in the context of future sugarcane plantation expansion. Breakthroughs in soil microbial community studies can reveal how microbes regulate plant growth and metabolism. By expanding exploration of soil microbial potential and deepening the concept of sustainable development, environmentally friendly biofertilizers can be the primary choice to achieve optimal production potential. Liu et al. (2022) found that the use of biofertilizers led to greater accumulation of beneficial microorganisms in the sugar cane rhizosphere soil. Meanwhile, the decrease in some pathogenic bacteria, such as *Leifsonia*, most likely inhibited or slowed the onset of sugarcane dwarf disease, promoting plant health. The exploration of soil microbial diversity on sugarcane plantation land in Indonesia is still not widely reported; therefore, this study profiles microbial diversity in these provinces using a metagenomics approach. Thus, the aim of this study is to gain knowledge about the comparison of microbial communities in two different sugarcane plantation areas.

Material and Methods

Soil and litter sampling

Soil and litter sampling was conducted in two sugarcane plantation areas, namely Bunga Mayang (BUMA), in Lampung province, and Cinta Manis (CIMA), in South Sumatra province. Soil sampling was conducted 1 week after sugarcane harvest. To analyze soil chemical and biological properties, soil samples were taken from depths of 0-20 cm and 20-40 cm. In addition, samples for *plant cane* (PC) and *ratoon cane* (RC) sugarcane fields were distinguished. Meanwhile, sampling of sugarcane debris scattered on the soil surface was carried out according to the PC and RC categories. For next-generation sequencing (NGS) analysis, composite soil samples taken from 5 locations, several points (blocks) representing one PC or RC plot were used.

Soil chemical analysis parameter

Soil samples were assayed for pH, organic matter, total nitrogen, total phosphorus, and total potassium (Balai Penelitian Tanah, 2009). The soil pH was obtained using a pH meter in a soil suspension made from dry soil and deionized water (water:soil = 2.5:1 (w/v)). The potassium dichromate volumetric method—outside heating method was then used to assay the soil organic matter (SOM) content, while the Kjeldahl method was used to determine the total nitrogen (TN).

Similarly, the total phosphorus (TP) content was determined with the NaOH alkali fusion–molybdenum antimony anti-spectrophotometric method, while total potassium (TK) was determined with NaHCO₃.

Soil biological analysis

After packing the samples in aseptic bags, they were immediately transported to the laboratory at low temperature up to 4 °C until processing. These soil samples were then divided into two parts: one for analyzing soil physicochemical properties and the other for storage at -80 °C until required for soil DNA extraction. Total microbial analyses were conducted by measuring total plate counts for bacteria and fungi, using NA and PDA medium, respectively. Fungal diversity was analyzed through Phytomicrobiome analysis using a Fungal ITS gene amplicon (subregions ITS1) with the help of services from Macrogen. The sample was previously extracted for DNA, then sequenced, and subjected to bioinformatics analyses.

Genomic DNA extraction and PCR amplification

Soil samples frozen at -80 °C were used for Phytomicrobiome analysis. First, DNA was extracted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082), followed by an initial quantification and purity assessment with the Nanodrop 2000 spectrophotometer (Thermo Scientific). For more accurate quantification, the Qubit dsDNA HS Assay Kits (Thermo Scientific) were used to measure DNA integrity and concentration. PCR amplification was performed using KOD Multi & Epi (KME-101), and the PCR products were visualized by agarose gel electrophoresis. Amplification and sequencing of the ITS1 region of the Fungal ITS gene was conducted using a primer 5'-GGAAGTAAAAGTCGTAACAAGG-3' (forward) and a primer 5'-GCTGCGTTCTTCATCGATGC-3' (reverse).

Library preparation, sequencing, and bioinformatics analysis

Library preparation was performed using kits from Oxford Nanopore Technologies. Oxford Nanopore Technology (ONT) enables long-read sequencing that captures the entire ITS region, delivering a fast, cost-effective, and high-throughput method. By including all informative sites within the ITS region, these full-length ITS sequences provide enhanced taxonomic and phylogenetic resolution for accurate fungal identification. Nanopore sequencing was managed

with MinKNOW (version 23.04.5), while basecalling was performed with Guppy (version 6.5.7) using a high-accuracy model (Wick et al., 2019). The quality of FASTQ files was visualized with NanoPlot, and NanoFilt was used for quality filtering (Coster et al., 2018; Nygaard et al., 2020). Classified reads were obtained using the Centrifuge classifier (Kim et al., 2016), with the fungi index built from the NCBI ITS RefSeq database (<https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/>). Further analysis and visualizations were conducted using Pavian (<https://github.com/fbreitwieser/pavian>), Krona Tools (<https://github.com/marbl/Krona>), and RStudio, utilizing R version 4.2.0 (<https://www.R-project.org/>).

Results and Discussion

Soil chemical and biological analysis of sugarcane fields in Bunga Mayang and Cinta Manis plantations

Ratooning is an agricultural technique in which monocotyledonous plants are harvested by removing the above-ground biomass while retaining the roots and shoot tips to allow regrowth in the following season (Bamisile et al., 2021; Fallah et al., 2023). This technique is often used on crops such as sugarcane, rice, and bananas, with sugarcane ratooning accounting for nearly 50% of global sugarcane production (Li & Yang, 2015). However, long-term ratooning often causes soil-related problems, including soil-borne diseases and increased soil alkalinity, mainly due to excessive use of agrochemicals (Diacono & Montemurro, 2011). Therefore, implementing effective soil management strategies is essential to address these challenges. Fertilization for optimal sugarcane growth was carried out twice for the PC category and once for the RC category. In the PC category, the first fertilization involved 200 kg ha⁻¹ of urea and 300 kg ha⁻¹ of TSP, while the second fertilization applied 250 kg/ha of Urea and 300 kg ha⁻¹ of KCl. In the RC category, fertilization was done once with 550 kg ha⁻¹ of Urea, 300 kg ha⁻¹ of TSP, and 300 kg ha⁻¹ of KCl. Fertilization is typically applied at a soil depth of 0-20 cm (topsoil), as this layer tends to have better soil fertility than deeper layers.

In addition, the implementation of Zero Burning in the BUMA and CIMA plantations has been ongoing for more than 5 years. This, of course, could affect the soil's chemical and biological properties in both plantations. Soil chemical and biological characterization was conducted in both plant cane (PC) and ratoon cane (RC) areas. The results of the soil properties analysis are presented in Table 1. Based on

the analysis, the soil in both plantations tends to be acidic with a pH of around 5-6. Meanwhile, there are differences in land conditions at BUMA and CIMA plantations. The CIMA plantation has higher C-organic and N-total values compared to the BUMA plantation. However, several other parameters, such as P₂O₅, K₂O, and microbial populations at the BUMA plantation, tend to be higher. In addition, the chemical and biological characteristics between PC and RC in plantations did not show any significant differences.

Soil pH has been reported as one of the main factors determining the structure of soil microbial communities under the control of different fertilizer applications (Bello et al., 2021). Liu et al. (2022) added that soil pH, total nitrogen, and available potassium were the main factors influencing bacterial community composition, while total soil phosphorus, available phosphorus, pH, and available nitrogen were the main drivers of fungal communities. Based on the results, the pH in the BUMA plantations was slightly more acidic than in CIMA. According to Li et al. (2012), the slightly higher acidity in conventional system soils may be due to the application of agrochemical inputs and intensive nitrogen use through fertilization. In addition, successive long-term cultivation of sugarcane leads to a decrease in soil pH. Soil pH can significantly alter the composition and function of the microbiota (Pang et al., 2021).

Litter is a biomass source in sugarcane plantations that enriches organic matter and is thought to play both direct and indirect roles in plant health and in interactions with soil microbial communities. In contrast to the soil analysis results, the litter produced by sugarcane on both plantations shows the same conditions, with C-organic around 47%, N-total around 0.7%, and a C/N ratio >60 (Table 2). Thus, it is known that the chemical characteristics of litter in the two plantations are similar.

Phytomicrobiome analysis of sugarcane plantations in Lampung and South Sumatra

All four samples (RC-BUMA, PC-BUMA, RC-CIMA, and PC-CIM) successfully generated 100,000 raw reads with mean read qualities ranging from Q14.1 to Q14.5, indicating comparable sequencing performance. Quality filtering improved mean read quality in all datasets (Q14.2–Q14.7) while reducing the total number of reads and mean read length. Read retention ranged from 53.2% (PC-CIMA) to 79.4% (RC-BUMA) (Table 3), reflecting differences in the proportion of reads meeting quality and length thresholds. RC-BUMA and RC-CIMA showed the highest retention rates and the smallest reductions in read length indicating better initial data quality.

Table 1. Soil characteristics of the BUMA and CIMA plantations

Location	Block	Category	Depth (cm)	pH	C-organic (%)	N-total (%)	P ₂ O ₅ (mg 100g ⁻¹)	K ₂ O (mg 100g ⁻¹)	Total bacteria (cfu gr ⁻¹)	Total fungi (cfu gr ⁻¹)
BUMA	075	RC	0 - 20	5.25	1.47	0.14	75.36	7.24	1.0 x 10 ⁷	2.0 x 10 ⁴
	075	RC	20 - 40	5.46	1.19	0.12	33.07	5.48	4.7 x 10 ⁸	6.0 x 10 ⁴
	133	PC	0 - 20	5.73	1.09	0.16	67.30	6.02	3.5 x 10 ⁸	7.0 x 10 ⁴
	133	PC	20 - 40	6.63	1.11	0.14	38.33	5.41	5.7 x 10 ⁷	1.0 x 10 ⁵
CIMA	III.7.120	RC	0 - 20	5.83	2.55	0.16	62.39	6.60	2.7 x 10 ⁸	5.5 x 10 ⁴
	III.7.120	RC	20 - 40	5.67	1.81	0.14	38.34	4.20	1.6 x 10 ⁸	1.0 x 10 ⁴
	III.7.155	PC	0 - 20	5.89	2.28	0.19	55.59	8.42	1.0 x 10 ⁷	2.5 x 10 ⁴
	III.7.155	PC	20 - 40	5.76	1.04	0.11	13.87	2.98	1.0 x 10 ⁷	1.5 x 10 ⁴

Table 2. Results of litter analysis at BUMA and CIMA plantations

Location	Block	Category	C-organic (%)	N-total (%)	C/N ratio
BUMA	075	RC	48.56	0.79	61.47
	133	PC	47.01	0.71	66.21
CIMA	III.7.120	RC	47.81	0.71	67.34
	III.7.155	PC	46.59	0.75	62.12

Table 3. Statistics data of reads

Sample	Before filtering			After filtering		
	Mean read length (bp)	Mean read quality	Number of reads	Mean read length (bp)	Mean read quality	Number of reads
RC-BUMA	836.9	14.1	100,000	805.8	14.4	79,444
PC-BUMA	822.0	14.5	100,000	736.0	14.7	70,113
RC-CIMA	819.8	14.2	100,000	801.1	14.4	78,539
PC-CIMA	892.1	14.1	100,000	748.4	14.2	53,237

Overall, filtering successfully removed lower-quality reads and produced high-quality datasets suitable for downstream analyses.

Based on the results, sugarcane plantations in BUMA (Lampung) and CIMA (South Sumatra) exhibit very high levels of diversity. Based on Figure 1a, it is known that there are nine genera that dominate the two plantations, namely *Protrudomyces*, *Lichtheimia*, *Spizellomyces*, *Phlyctochytrium*, *Coralloidiomyces*, *Delfinachymtrium*, *Coccomyces*, *Myxozyma*, and *Brettanomyces*. Of the 9 Genus, there was a very significant difference in fungal dominance between BUMA and CIMA. *Protrudomyces*, *Coralloidiomyces*, and *Spizellomyces* were the three main genera that dominated the BUMA plantation, while the CIMA plantation was dominated by *Lichtheimia*, *Phlyctochytrium*, and *Spizellomyces*.

Technical culture and environmental stress can affect soil microbial diversity. Malviya et al. (2021) stated that the highest number of operational taxonomic units (OTUs) of bacteria and fungi was found in the sugarcane-legume intercropping system. In addition, intercropped sugarcane plants showed higher plant biomass than monoculture plants. Liu et al. (2022) revealed that bio-fertilizers increased the abundance of beneficial bacteria in the sugarcane rhizosphere and reduced pathogenic bacteria (e.g., *Leifsonia*), potentially slowing or suppressing disease development. Results of a metagenomics study revealed that in periodically waterlogged fields, the relative abundances of *Chloroflexi*, *Actinobacteria*, and *Basidiomycota* increased, while those of *Acidobacteria* and *Ascomycota* decreased (Leelastwattanagul et al., 2023).

Further analysis revealed fungal species-level diversity in BUMA and CIMA (Figure 1b). Based on the analysis, it is known that BUMA plantation is dominated by *Protrudomyces lateralis*, *Coralloidiomyces digitatus*, and *Lichtheimia corymbifera*, while CIMA plantation is dominated by *Lichtheimia corymbifera*, *Phlyctochytrium californicum*, and *Spizellomyces plurigibbosus*. Thus, it is known that microbial diversity differs at both the genus and species levels between BUMA and CIMA. If a breakdown is made for each experimental land with several sugarcane categories (Figure 2), the results show that in the RC category, *Lichtheimia corymbifera* is the most dominant species in both BUMA (RC-B) and CIMA (RC-C) plantations. According to Morais et al. (2018), *L. corymbifera* is a mesophilic fungus that can produce β -glucosidase up to 39 U/g dry substrate (or 3.9 U/mL) with optimum pH and temperature of 4.5 and 55 °C, respectively. The enzyme produced can remain stable for 1 hour at 45 °C. However, in the PC category, BUMA (PC-B) is dominated by *P. lateralis*, meanwhile CIMA (PC-C) is dominated by *P. californicum*. Based on the graph, the lands in each location have different biodiversity. The differences in microbial biodiversity, as suggested by recent findings, could be one of the factors influencing plant growth and especially plant health. Microbial diversity can directly or indirectly affect the nutrient cycle, supplying nutrients to plants.

Analysis of the microbial community on a land can serve as a baseline for describing land suitability and assessing the quality of land ideal for sugar cane cultivation. Zhang et al. (2020) showed that across different carbon source assimilation conditions, the phylum *Ascomycota* was the most pronounced biomarker microbial community. Liu et al. (2022) added that the microbial genera contributing to the variability between fertilizers included the bacteria *Anaerolineae*, *Vulgatibacter*, and *Paenibacillus*, and the fungi *Cochliobolus*, *Sordariales*, and *Dothideomycetes*. In addition, Leelastwattanagul et al. (2023) added that beneficial microbes such as *Arthrobacter*, *Azoarcus*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Streptomyces* thrive in normal soils, potentially serving as biomarkers for ideal soil conditions. In contrast, phytopathogens and growth-inhibiting bacteria were prevalent in regularly waterlogged fields, indicating unsuitable conditions.

Technical culture in sugarcane cultivation, especially fertilization practices, plays an important role in shaping the diversity and composition of the soil microbial community. Based on the results, the

fungal community in *Alpha diversities* (Figures 3 and 4) obtained from several indices (*Observed*, *Chao1*, *ACE*, and *Shannon*) states that there is a difference in the value of the diversity index in sugarcane fields in CIMA compared to BUMA plantation (PC and RC categories). The diversity and abundance of fungi in the CIMA land are higher than in the BUMA plantation. When compared from the soil chemical characteristics aspect, namely C-organic. It shows that the CIMA has a higher value than that in BUMA plantation, so that the source of carbon (C), which is a source of energy or food for soil microbes, is more abundant. It results in more microbes being able to grow and develop well in the CIMA. However, the alpha diversity analysis between BUMA and CIMA plantations showed no significant difference. Previous research found no difference in alpha diversity between drained and flooded agricultural fields (Breidenbach et al., 2016; Gschwend et al., 2020). Monela et al. (2022) reported that the maintenance system with organic and conventional fertilizers showed a very similar alpha diversity index, characterized by the dominance of growth-promoting microbes, including bacteria from the genera *Bacillus* and *Bradyrhizobium*, and the fungal genus *Trichoderma*.

The relative abundance analysis of the phytomicrobiome (Figure 5) shows the distribution of fungi in the BUMA and CIMA plantations. Many types of fungi are developing in the soil. However, what is interesting in the phytomicrobiome analysis of the experimental land is that one of the fungi expected to be found in the soil, *Trichoderma*, has a very low distribution, accounting for approximately 1% of the total fungi. The *Trichoderma* found on both plantations is presented in Figure 6. *Trichoderma* plays many roles, supporting sugarcane growth and acting as a biological agent to address fungal diseases. Liu et al. (2022) reported that the application of biofertilizers and compound fertilizers in sugarcane plantations significantly increased bacterial diversity but did not significantly affect the fungal community. This is thought to be caused by the application of bio-fertilizers and compound fertilizers that have specific effects on the rhizosphere environment, and the functional bacteria in bio-fertilizers may increase the secretion of certain chemicals from sugar cane while affecting the interaction of these chemicals with the rhizosphere community, thereby affecting the entire root-soil-microbe system.

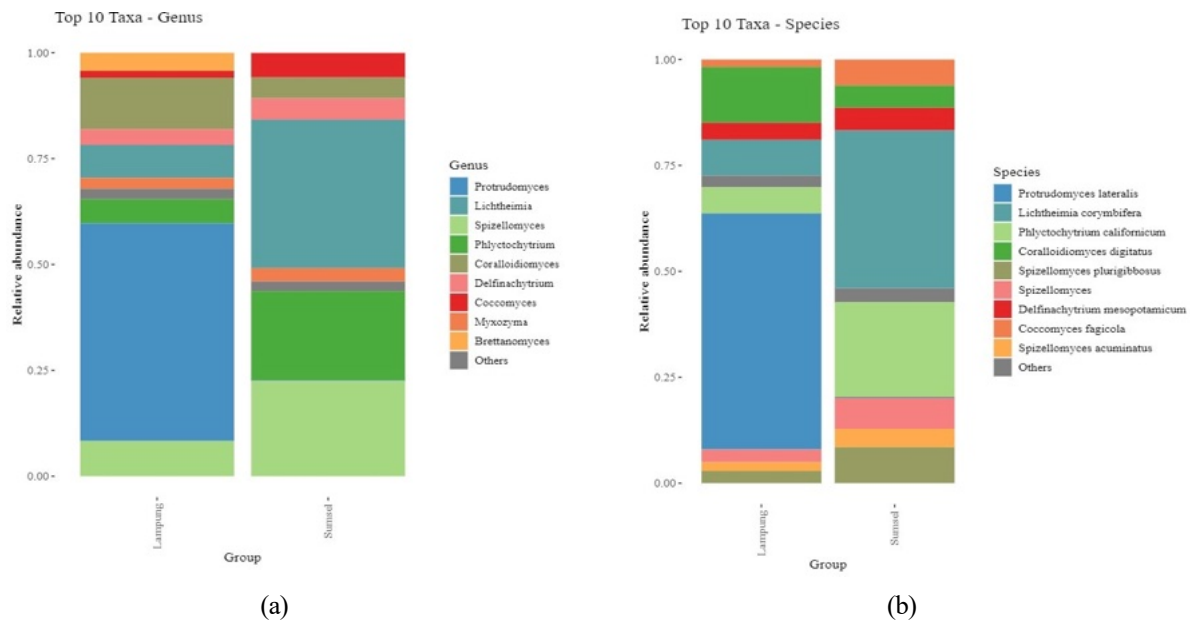


Figure 1. Fungal composition dominating BUMA (Lampung) and CIMA (South Sumatra) plantation at Genus (a) and Species (b) levels.

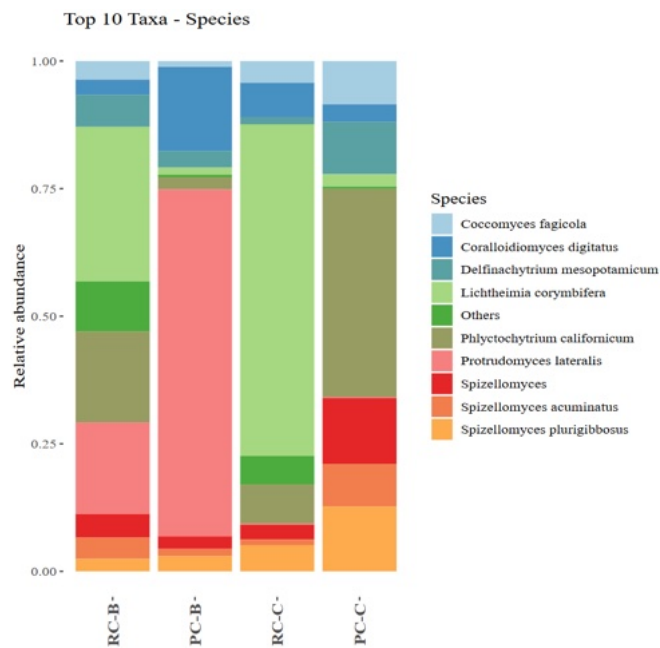


Figure 2. Fungal composition dominating of PC and RC categories at BUMA (B) and CIMA (C) plantation at species levels

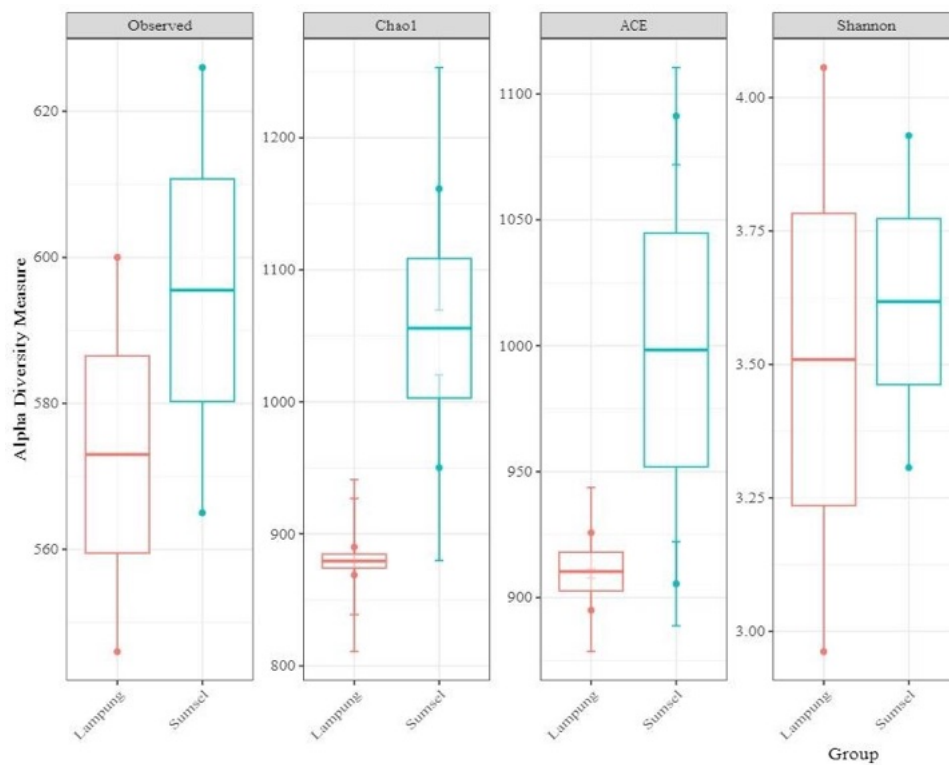


Figure 3. Box Plot of Fungal *Alpha Diversities* at BUMA (Lampung) and CIMA (South Sumatra) Plantations.

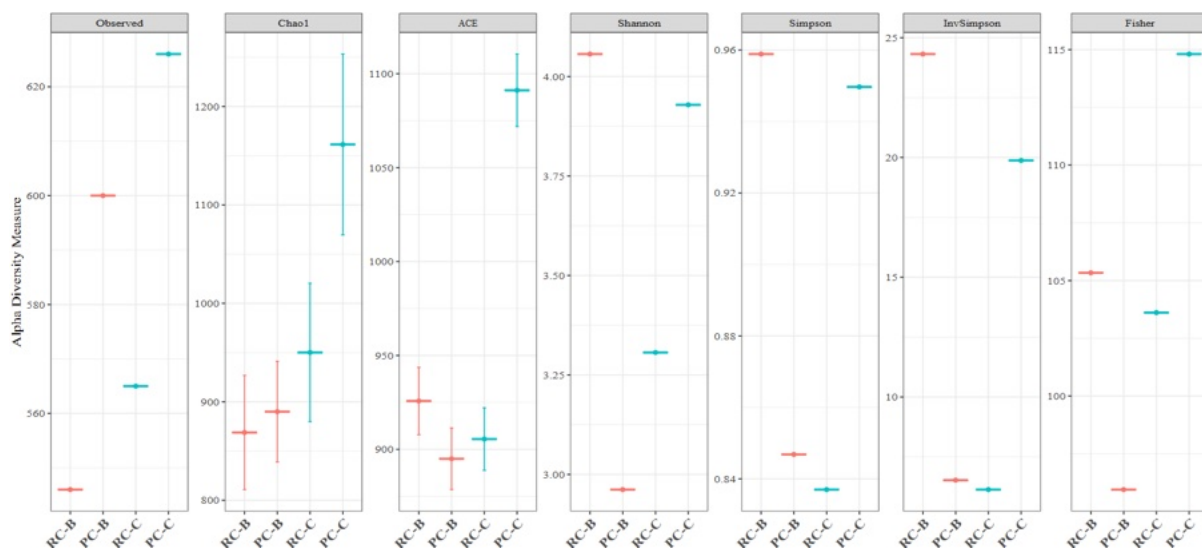


Figure 4. Box Plot of Fungal *Alpha Diversities* PC and RC categories at BUMA (B) and CIMA (C) Plantations.

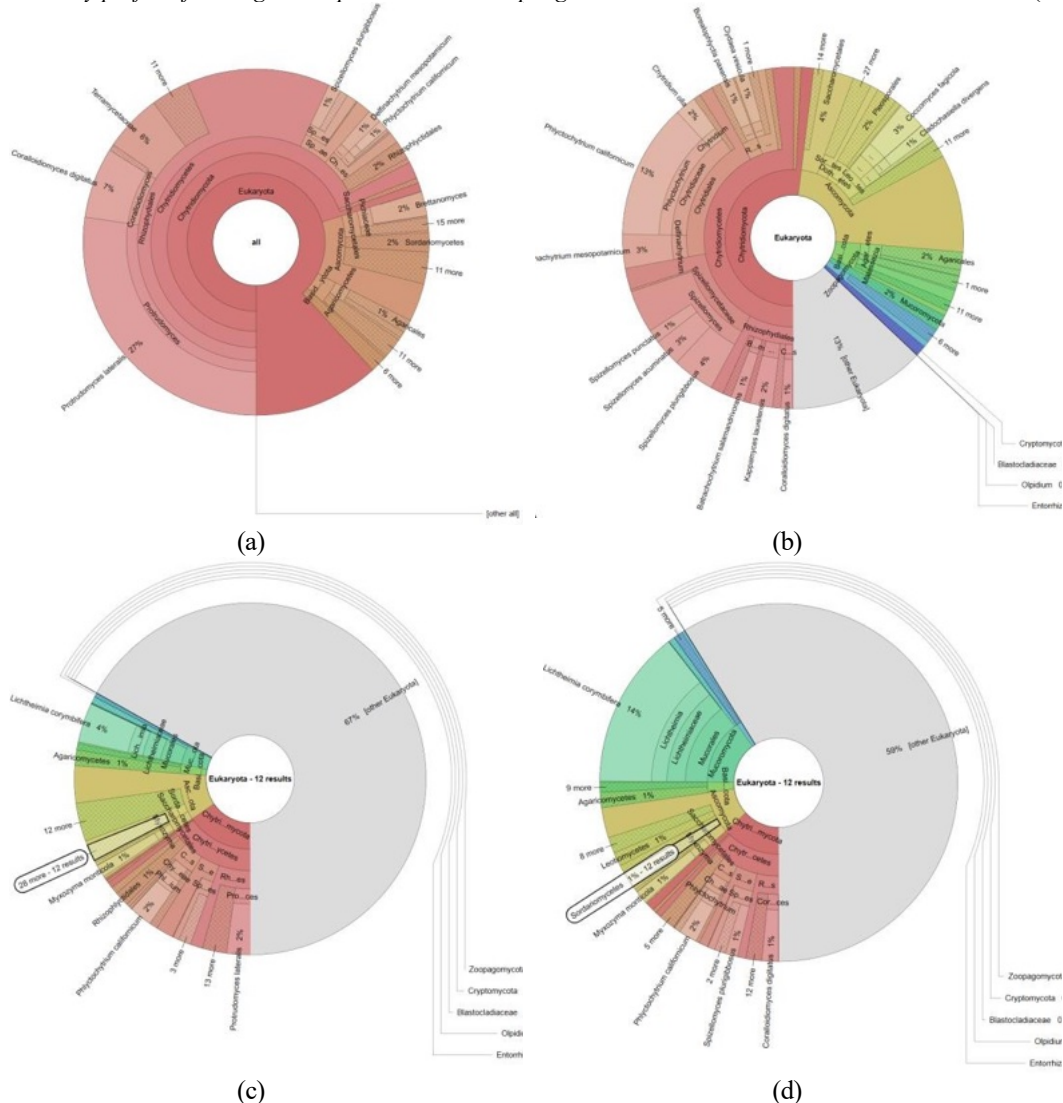
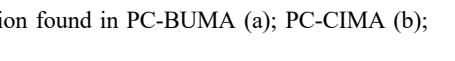


Figure 5. Next Generation Sequencing analysis results of the distribution of soil microbes (fungi) found in the PC-BUMA (a); PC-CIMA (b); RC-BUMA (c) and RC-CIMA (d).

The application of *Trichoderma*-based biofertilizers altered the pasture microbial community, and *Trichoderma* abundance became the primary driver of pasture biomass production. This suggests that the addition of biofertilizer alters a series of biological processes at the rhizosphere level (Zhang et al. 2018). Meanwhile, the fungal diversity under compound fertilizer treatment appears to have a greater effect on the biological processes of rhizosphere fungi. This phenomenon may be due to the interplay between fertilizer and microbial effects, which needs to be further explored (Bello et al., 2021).

The findings from soil samples in the BUMA and CIMA plantations show that many soil microbes are growing and developing in the sugarcane plantation areas. These soil microbes have the potential to be

used to enhance soil fertility, protect plants, provide decomposer isolates, and confer several other benefits. These microbes require energy or a food source to continue growing and reproducing, which comes from organic matter. The organic matter in sugarcane plantations generally comes from sugarcane litter and molasses, and the Zero Burning technology can increase the availability of these food sources for soil microbes, making the soil more fertile and optimal as a growth medium for sugarcane plants. Galitskaya et al. (2017) reported that the increase in the *Saccharomycetales* group following biofertilizer application can lead to the synthesis of active compounds that promote root growth and cell division and provide the substrate needed for the proliferation of other effective microorganisms.



stresses, including their effects on nutrient conversion (Singh et al., 2013; Liu et al., 2022). The importance of rhizosphere microbes as neighbors of plant roots for plant health and growth cannot be overstated (Gunarto, 2000; Pang et al., 2021). Rhizosphere microbial communities can promote the growth of plant above-ground tissues by enhancing adaptation to environmental stresses, improving nutrient acquisition, and improving plant metabolic functions. A study demonstrated the defense response of a rhizosphere microbial community consisting of *Pseudomonas*, *Trichoderma*, and *Rhizobium* strains to specific biotic stresses in chickpea (Singh et al., 2013). In addition, it is shown that plants can defend

themselves against herbivore attack through self-defense mechanisms that recruit beneficial plant-promoting rhizobacteria/fungi (Yi et al., 2009).

Conclusion

The study revealed differences in microbial dominance between sugarcane plantations in Lampung and South Sumatra. Sugarcane fields in Lampung were dominated by the genus *Protrudomyces*, *Coralloidiomyces*, and *Spizellomyces*, while South Sumatra was dominated by the genus *Lichtheimia*, *Phytochytrium*, and *Spizellomyces*. At the species level, *Lichtheimia corymbifera* was the most dominant species in the RC category in both the BUMA (RC-B) and CIMA (RC-C) plantations. However, in the PC category, BUMA (PC-B) was dominated by *Protrudomyces lateralis*, meanwhile CIMA (PC-C) was dominated by *Phytochytrium californicum*.

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