

Rhizosphere Microbial Community Structure of Transgenic versus Non-Transgenic Maize Varieties

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Abstract

The soil rhizosphere community is an important indicator of the overall health of the plant and the resulting yield at harvest. The study determined the rhizosphere microbial community structure of transgenic and non-transgenic maize plants. A farm plot was divided into 4 sections for transgenic maize (SAMMAZ 74T) and non-transgenic variety 8325-8. The maize was infected at the tasseling stage, and phenolic content was determined after 10 days. The microbial community indices were subsequently determined using metagenomic tools. The physicochemical parameters and microbial population of the soil for maize 8325-8 and SAMMAZ 74T were not significantly different at $P < 0.05$. The soil with infected maize was the most diverse, with a Shannon index of 1.834. The soil with infected maize was the most diverse, with a Shannon index of 1.834. The transgenic maize SAMMAZ 74T had the highest dominance of bacteria (0.2536) and fungi (0.10930). Proteobacteria were the predominant bacterial phylum, while Ascomycota was the predominant fungal phylum. The study concludes that the non-transgenic maize had a better contribution to the soil microbial community than the transgenic maize variety.

Keywords: Rhizosphere, diversity, community, transgenic, metabolites

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Introduction

The impact of phytopathogenic fungi on agriculture remains a significant concern, as it causes substantial decreases in crop yield that lead to economic as well as nutritional losses [1]. The virulent strains of these fungi present a huge risk to global food supply and security. These fungi encompass a diverse range of species that can induce various plant diseases, such as smut, rust, wilting, powdery mildews and damping-off. The phytopathogenic fungi employ mutual mechanisms to infect various parts of a plant to establish infection, characterized by symptoms and deformities. Infecting fungi establish communication with their plant hosts through the utilization of effector proteins that sustain the infection process. The most prevalent categories of fungi are Fusarium species, Phytophthora, Downy and powdery mildews, smuts, and rust fungi. Among the phytopathogenic fungi, Fusarium and Aspergillus species are major fungal pathogens of maize, causing ear rots and producing mycotoxins with deleterious effects [2, 3]. These fungi primarily enter maize kernels through the silks, with *F. graminearum*, *F. verticillioides*, and *A. flavus* being the most significant threats [3]. Other identified fungal species

contaminating maize grains include *F. moniliforme*, *A. flavus*, and *A. parasiticus*, among others [2].

Transgenic plants were introduced and widely adopted because of their comparative advantages in terms of increasing yield, reduction of field and post-field losses, development of resistance barriers against pests, diseases, and weeds, and increasing crop varieties, among others. Some members of the public have voiced serious concerns about eating transgenic food and eating genetically modified organisms (GMOs). These concerns, originating from religious groups and ethical considerations, revolve around the perceived unnaturalness of GMOs and the associated fears of death, cancer, and organ failure [4]. Because of their encouragement of monoculture farming, which eventually results in the loss of more organic and native seed varieties, genetically modified organisms (GMOs) have a severe effect on food production systems and biodiversity.

The fitness of transgenic plants and their hybrids with wild types is crucial in the assessment of the ecological risks it imposes on sustainable agriculture. Fitness assessment is essential for modelling and planning transgenic organism release programs, typically



involving direct comparisons of the fitness parameters [5]. While most studies indicate reduced fitness in transgenic strains, some hybrids may exhibit enhanced fitness under certain conditions, highlighting the need for comprehensive evaluation to mitigate potential ecological risks associated with transgene escape [6, 7]. Factors such as thermal environments, fungicide resistance, and melanization referred to as local adaptation in fungi plays a crucial role in shaping host-pathogen interactions and evolutionary trajectories [8]. Transgenic or genetically modified approaches offer promising solutions for plant disease resistance without compromising fitness, with success recorded both in the laboratory and on the field [9]. While fungicide resistance can lead to fitness penalties, the extent varies depending on environmental factors and pathogen life cycle stages [10].

Materials and Methods

Experimental design and land preparation

Experimental site

The plot was located in the Federal University of Lafia Agricultural Experimental Farm, located between longitude 8°28'21" and latitude 8°34'43", Nasarawa State, Nigeria. The maize grains were transgenic drought-resistant SAMMAZ 74T and non-transgenic yellow maize 8325-8. The experimental plots were laid out in a Completely Randomized Design (CRD). The plot (40 ft x 40 ft) was divided into four sections, separated by 8 ft from each other to prevent cross-contamination or exchange through the soil. Sections A and B contain 8325-8 breed, and sections C and D contain SAMMAZ 74T.

Soil sampling

Soil samples were collected thrice in the course of the study. The first sampling was done before planting to determine the culturable microbial population and physiochemical characteristics of the soil. The second collections were 65 days after planting (tasselling stage) to quantify enzyme activities, and lastly, seven days after infection with the fungal pathogen.

Determination of culturable microbial population

To determine the microbial load in the soils, 100 mg of the soil was added to 900 mL of sterile distilled water, and the mixture was put on a rotary shaker at 200 rpm for 20 min. The mixture was further diluted to 10^{-9} , and an aliquot was plated using the pour plate method on Nutrient agar for bacterial population and 10^{-4} plated on PDA to which chloramphenicol (50 µg/mL) was added. They were incubated at 28 °C for 24 h to 48 h (bacteria) and 25 °C for 3 – 7 days (fungi), after which the colony-forming units were counted and recorded.

DNA extraction from soil

Genomic DNA was extracted from the rhizosphere soil in replicate (Inqaba Scientific, Ibadan, Nigeria). Fresh soil (1 g) was used for the DNA extraction. Power Soil DNA isolation kit was used for extraction following the manufacturer's instructions (Mo Bio Laboratories,

Solana Beach, CA, USA). DNA purity and quantity were confirmed using agarose gel electrophoresis and a Nanodrop spectrophotometer (Thermo Fischer Scientific, CA, USA). The obtained genomic DNA were normalised with ddH₂O to a dilution of 1 ng/µL) after which it was amplified using primer 338F/806R (338F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT) targeting bacterial 16S rDNA V₃ to V₄ regions. The fungal ITS region was amplified with primer pair ITS1F/ITS1R (ITS1F: CTTGGTCATTTAGAGGAAGTAA, ITS1R: GCTGCGTTCATCGATGC). Using the modified methods of Shen *et al.* [11], the PCR mixtures were treated to an initial denaturation at 95 °C for 3 min, followed by 28 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s and elongation at 72 °C for 45 s. The products were extracted from a 2 % agarose gel and purified using a PCR clean-up kit (Qiagen) to remove primer dimers and non-specific fragments. The obtained amplicons were pooled to form a composite sequencing library at equal concentration. The library preparation involved ligation adapters as described by Illumina Inc.

Illumina Hiseq sequencing and data processing:

Sequencing was performed on Illumina Hiseq to obtain high-throughput paired-end reads. Low-quality reads were trimmed, and overlapping paired-end reads merged using FLASH to reconstruct full-length amplicons. Chimeric sequences were identified and removed with UCHIME. High-quality reads were clustered into operational taxonomic units (OTUs) based on similarity using UPARSE. Taxonomic assignment was performed against 16S rRNA genes for bacterial and fungal ITS sequences using BLASTN.

Statistical analysis

All experiments were replicated and results reported as mean ± standard deviation (SD). The microbial community structure and richness were determined with α -diversity indices. The heatmap was used to determine the relationship between phenolic compounds and infected maize.

Results and Discussion

The mean bacterial and fungal populations (Table 1) were generally high in the experimental soils, though the CFU/g values obtained were not statistically significant at $p < 0.05$.

Table 1: Culturable bacterial and fungal populations in the experimental soils

Parameter	SAMMAZ 74T Planted Soil	8325-8 Planted Soil
Bacterial Population(x 10 ⁷ CFU/g soil)	6.30 ± 1.22 a	4.78 ± 1.23 a
Fungal Population (x 10 ⁴ CFU/g soil)	2.12 ± 0.98 a	3.52 ± 1.13 a

Result is presented as Mean ± SD. ab = Letters following mean indicate samples are significantly different at $p < 0.05$ (Tukey HSD all-pairwise comparisons test)

A total of 78 genera were shared by rhizosphere fungal communities irrespective of the health status of the sampled plants, while the bacteria shared 199 together (Fig. 1). The rhizosphere of control maize 8325-8 (HNTM) had nine unique fungal genera (*Syncephalastrum*, *Sebacina*, *Sporobolomyces*, *Cokeromyces*, *Thermothielavioides*, *Preussia*, and three unidentified genera) which were unique to the soil, while the infected 8325-8 (INTM) plant rhizosphere had three (*Thamnostylum*, *Auriculariopsis* and an

unidentified genera). The control (HTM) and infected (ITM) transgenic maize (SAMMAZ-75T) had no genus peculiar to either of their rhizosphere soils. Eight genera were found shared by all the soils except those of infected 8234 (INTM). The bacterial community had three genera (*Sinosporangium*, *Arthrobacter*, and *Nocardiopsis*) peculiar to the control soil (HNTM), and one genus (*Adhaeribacter*) in the transgenic control (HTM) soil.

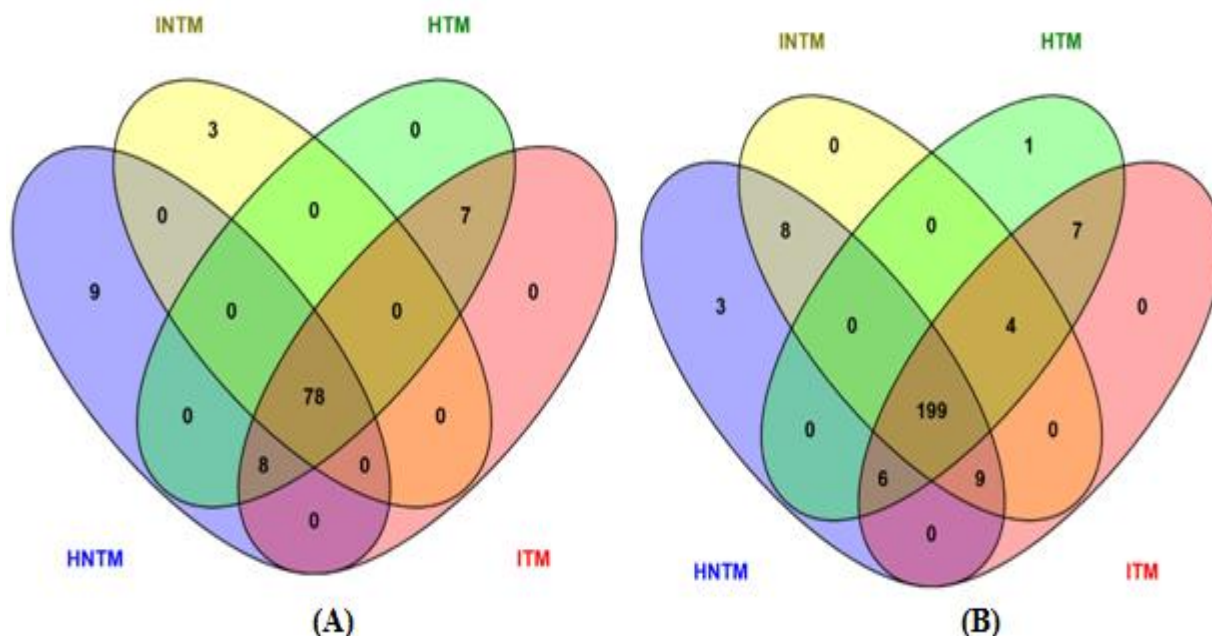


Figure 1: Venn diagram of the (A) Fungal genera and (B) Bacterial genera in the rhizosphere soil communities

Quality reads obtained in the study for the bacterial communities ranged between 164,503 (ITM) and 217,586 (HNTM), and the fungi had 41,857 (ITM) and 79,927 (HTM) (Table 2). The total number of OTUs observed was the highest, 17,536 (bacteria) and 622 (fungi), at 97 % similarity. The soil with infected 8325-8 maize plant (INTM) had the highest diversity (Shannon diversity: 1.834), lowest dominance and was evenly distributed, while the soil with infected SAMMAZ 74T was the most dominated, with the lowest diversity and richness, and poorest in bacterial distribution. All the bacterial and fungal communities harboured unseen diversities, though with < 1 % abundance. The fungal community in the infected soils (INTM and ITM) were the richest. Fungal community in the infected 8325-8 soils (INTM) was the most diverse and least dominated, while soils with SAMMAZ 74T had the lowest diversity and highest dominance. All communities are highly evenly distributed. Soil INTM was the least dominated (0.0898), and HTM (0.1093) was the most dominated. The soil INTM had the highest diversity (2.446), while HTM (2.257) had the lowest diversity.

Table 2: Comparative diversity analysis between the different rhizosphere soils

		HNTM	INTM	HTM	ITM
Bacteria	Quality reads	217,586	193,427	184,729	164,503
	OTUs	17,536	10,169	19,416	12,902
	Shannon H'	1.774	1.834	1.725	1.600
	Dominance-D	0.2339	0.2027	0.2536	0.2784
	Evenness	0.5361	0.5687	0.5103	0.5004
Fungi	Quality reads	68,375	72,513	79,927	41,857
	OTUs	403	622	589	494
	Shannon H'	2.359	2.446	2.257	2.411
	Dominance-D	0.09786	0.08981	0.10930	0.09562
	Evenness	0.9619	0.9618	0.8907	0.9044

The richness of the taxa was identical across all the soils sampled, but differences were recorded in the abundance (Fig. 2). Proteobacteria were the dominant phylum in all the soil collected, with the least abundance of 32.34 % (INTM) and the highest abundance from ITM (44.07 %). Proteobacteria, Firmicutes, Acidobacteria and Actinobacteria were the most abundant phyla across all groups, jointly making up about 79.78 to 84.67 % of the bacterial community. The phylum Firmicutes increased in soils planted with infected transgenic maize (12.68 %) as compared with the healthy soil (9.88 %). Several of the phyla, such as



Verrucomicrobia, Chloroflexi, Nitrospira, Planctomycetes and Gemmatimonadetes occur in very small fractions (< 4 %) but showed consistent presence. All the soil harbours a broad range of phyla (> 11), but the relative proportion differs depending on the plant

status and health. In the fungal communities, Ascomycota was the highest occurring phyla ranging from 80.78 to 87.12 %, while Mucoromycota had the lowest abundance (0.55 – 2.05 %).

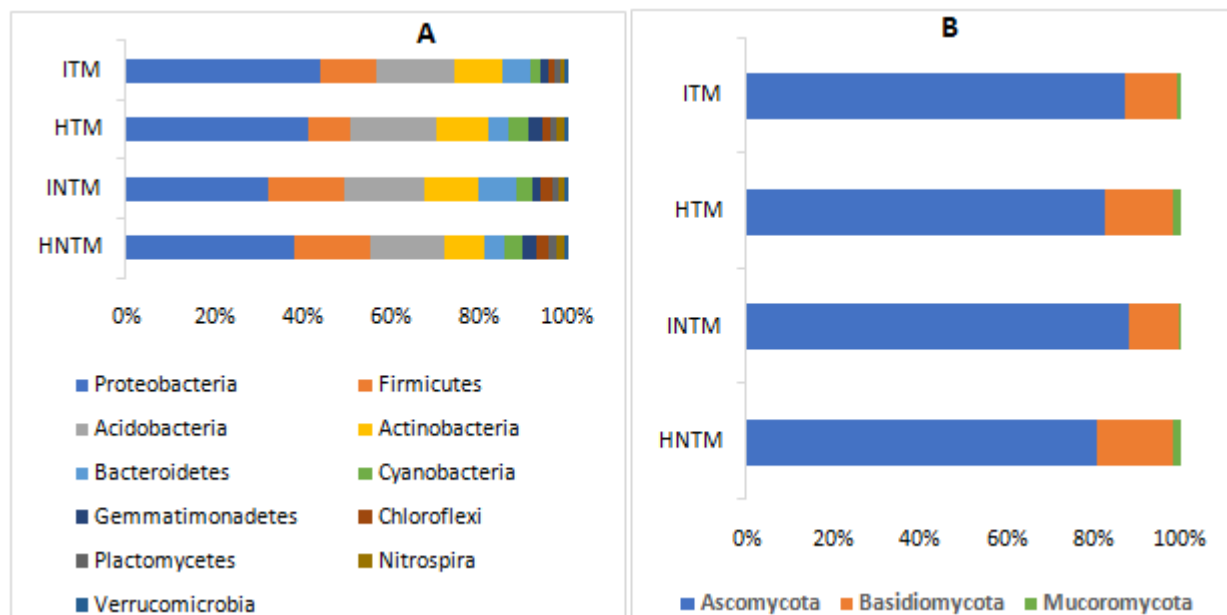


Figure 2: Relative abundance of the predominant bacterial and fungal phyla in the rhizosphere soil communities

Infection of the maize plant was carried out at the stage of tasselling. At this stage of development, the plants were more sensitive to stress, and are in high demand for nitrogen, phosphorus and potassium. It is also the stage in which the yield is decided.

The microbial population in the soils varied in their utilization of carbon according to the plots. While a gradual increase was recorded across all soils, the rhizosphere soil with transgenic plants had relatively higher microbial load compared to the non-transgenic group, suggesting microbial community difference in the utilization of carbon sources. The attributable reasons could include soil quality, pollution, organic matter, root exudates, plant litter, stress, microbial diversity, and plant growth stage [12]. The microbial load was relatively higher in the soils containing control maize plants (HNTM) compared to the infected groups, which suggests promotion of microbial functional diversity or consortium, especially in the early to mid-phases between 48 and 96 h. The microbial communities got saturated at 120 h, forming a plateau.

The transgenic lines reduced distinctiveness among the fungal genera. Eight genera were shared by all the soil fungal communities except the infected 8325-8 (INTM) maize, possibly as a defence mechanism to eliminate lines with unfavourable traits. The shared fungal genera (78) showed a conserved core set that does not change regardless of infection or changes in the soil biotic or abiotic environments. The variation recorded in microbial diversity and composition could result from the crop planted, environmental influence and type of

farming inputs applied [13-15]. Our study disagreed with Shen *et al.* [11] that transgenic plants improve the diversity of the soil bacterial community. The soil bacterial community structure showed a dominance from the phyla Proteobacteria, Firmicutes, Acidobacteria and Actinobacteria, which are typical in soil and plant rhizosphere [16]. The balance between Proteobacteria and Firmicutes was the most variable feature across the four soils, suggesting that infection and probably genetic manipulation influence the dominance of these two major phyla. Proteobacteria is linked with nutrient cycling and fast-growing (copiotrophic) communities. On the other hand, Firmicutes enrichment may indicate adaptation to stress and organic matter degradation. The presence of diverse minor phyla in the bacterial communities shows functional redundancy and resilience in the microbial ecosystem.

Ascomycota, with over 80.78 %, was the major phylum and the dominant fungal group in the four soils. These organisms play an important role in decomposition and nutrient cycling and have the capacity to adapt to varying environmental stress conditions and utilize diverse substrates in the rhizosphere environment. Mucoromycota, with their low abundance, will have a limited ecological role, such as niche management, while their low abundance could be a result of competitive exclusion by the dominant fungi. The genotype of the plant and treatment methods could alter the composition of microbial phyla, which did not align with the present study [17]. Finding showed no

significant differences in the phyla composition from the maize plots, but changes were noticed in the abundance of the different phyla. The study opined that with time, the microbial composition shall converge or stabilize to form a new equilibrium.

Conclusion

Finding in this research showed that non-transgenic plants accommodate better soil microbial communities, where interactions are probably mutual. The microbial communities are mainly dominated by the Ascomycota and Proteobacteria, while other phyla, though in lower percentages, also contribute to ecological dynamics in the root environment. The differences observed in the study were primarily between healthy and infected plants. The obtained difference between transgenic and non-transgenic maize plants was only significant in the enzyme activities and carbon utilization.

Conflicts of interest: No conflict of interest was declared by the authors.

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