

Microbiological evaluation of the rhizosphere of white Guinea Yam (*Dioscorea rotundata* L.), Maize (*Zea mays* L.) and Cassava (*Manihot esculenta*) in a tropical environment southwest Nigeria

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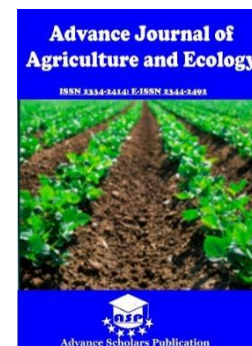
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Dioscorea rotundata, microbial diversity, nitrogen-fixing bacteria, *Manihot esculenta*, rhizosphere, sustainable agriculture, *Zea mays*

Abstract: The rhizosphere is a complex, dynamic environment where each plant species exerts a unique influence on the microbial communities that inhabit its rhizosphere. This study evaluates the microbial populations in the rhizospheres of three staple crops; yam (*Dioscorea rotundata*), maize (*Zea mays*), and cassava (*Manihot esculenta*) cultivated in southwest Nigeria. Soil samples were randomly collected from three distinct agricultural fields located at Atan (Ogun state), Ojo (Lagos state) and Ibadan (Oyo state) each representing unique soil types and environmental conditions. Soil sampling was conducted at a depth of 0-30 cm using sterile soil augers during the crops' peak vegetative stages to capture active microbial communities. The collected soil samples were made into composite samples and transported to the laboratory in black bags for analysis. Microbiological analysis was achieved using serial dilution technique while the rhizospheric bacteria and fungi species were cultured on media; Nutrient Agar (NA) and Yeast Extract Mannitol Agar (YEMA) for bacterial isolates with YEMA being selective for nitrogen-fixing bacteria while Sabouraud dextrose agar (SDA) was used for fungal isolates. The soil dilutions (10⁻⁵) were inoculated onto the media incubated at 26±2°C for 48 hours for bacteria and 3-5 days for fungi respectively. The microbial isolates were characterized with series of

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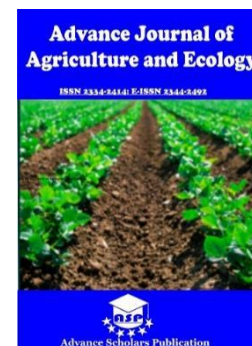
*biochemical tests and 16S rRNA molecular analysis. The results indicated significant variations in microbial communities in the rhizospheres of cassava, yam and maize which suggested that factors such as root exudates, nutrient uptake mechanisms, soil type, and plant growth conditions determined the rhizosphere microbiome that subsequently impacted on the level of soil fertility. A comparative analysis of cassava, yam and maize rhizosphere was carried out and nitrogen-fixing bacteria were highest at Atan (4.2×10^{-6} cfu/mL), heterotrophic bacterial population was highest at Ojo (4.1×10^{-6} cfu/mL) and heterotrophic fungal population was highest (3.7×10^{-6} cfu/mL) at Ibadan. Cassava rhizosphere (Atan) exhibited a higher concentration of nitrogen-fixing bacteria, while the rhizospheres of yam and maize (Ojo and Ibadan) displayed greater fungal diversity. The microbial community composition detected by random molecular characterization of selected isolates revealed *Aspergillus japonicus* (GenBank Ascension number: LC496497.1), *Penicillium simplicissimum* (GenBank Ascension number: KR296862.1), *Trichoderma harzianum*, *Fusarium* sp., *Agrobacterium tumefaciens* (GenBank Ascension number: MF767518.1), *Rhizobium rhizolycopersici* (GenBank Ascension number: NR_181268.1), *Bacillus pumilus* (GenBank Ascension number: EF491624.1), *Bacillus subtilis* (GenBank Ascension number: AB862127.1) and *Staphylococcus aureus* (GenBank Ascension number: CR029751.1). These findings underscore the importance of microbial communities in promoting nutrient uptake, ecological resilience among the three staple crops, enhancing disease - resistance, improving soil fertility and ecological function as biocontrol agents. This study highlights the potential for microbial interventions in sustainable agriculture and the synergy of organic agriculture in contributing to enhanced crop productivity in the region.*

INTRODUCTION

The rhizosphere, a thin layer of soil surrounding plant roots, is a biologically active zone where plant roots interact closely with a complex community of microorganisms. This zone is fundamental to plant health and productivity, influencing nutrient uptake, disease – resistance and overall soil fertility. Microbial populations in the rhizosphere, which include bacteria, fungi, archaea and protozoa, play critical roles in nutrient cycling, promoting plant growth, and

protecting plants from pathogens (Hinsinger *et al.*, 2011; Chepsergon & Moleleki, 2023). Therefore, mediating the plant-microbe interactions in soil is an ideal sustainable strategy for high crop yield (Shi *et al.*, 2024). Understanding the dynamics of this microbial community is crucial for developing sustainable agricultural practices, especially for key staple crops such as cassava, yam, and maize, which are vital to food security in many tropical and subtropical regions because the growth of

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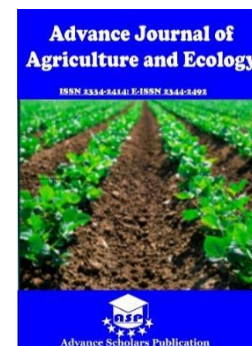


terrestrial plants and soil microbes is tightly cohesive (Trivedi *et al.*, 2020; 2021). It has been reported that the structural and functional diversity of rhizosphere microbial populations is determined by differences in root exudation and rhizode-deposition in different root zones and in relation to soil type, plant species, growth stage, cultural practices, such as tillage and crop rotation and other environmental factors (Lupwayi *et al.*, 1998).

However, the diversity and community structure of bacteria associated with the white Guinea yam (*Dioscorea rotundata*), the most cultivated and economically important yam in West Africa, have not yet been investigated (Idowu *et al.*, 2024). Yam (*Dioscorea* sp.), currently categorized as one of the top-ranked orphan crops (Kennedy, 2003), is a multi-species tuber crop belonging to the family *Dioscoreaceae* that is widely cultivated in Africa, Asia, parts of South America, the Caribbean, and the South Pacific Islands (Asiedu & Sartie, 2010). Approximately, 77 million metric tons of fresh yam tubers are produced annually worldwide, with Africa contributing approximately 98 % (FAOSTAT, 2022). West Africa produces more than 90 % of the global yam and is generally regarded as the world's yam belt region (FAOSTAT, 2022). In this region, white Guinea yam (*Dioscorea rotundata* L.), the predominant cultivar, plays a critical role in the human diet and sociocultural

systems (Asiedu and Sartie, 2010). It is an essential ingredient in government policies on food security and poverty alleviation as well as serves as a source of foreign exchange and income generation for people in this region (Scott *et al.*, 2000). Interestingly, annual yam production over the last three decades surged at a growth rate of approximately 3.75 %, it is expected to increase over the next two decades by an additional 3 %, mainly from using landraces with fewer chemical inputs but expanded land cultivation which is no longer sustainable owing to global population increase and cattle ranching activities (Scott *et al.*, 2000). Meanwhile, the substantial surge in yam productivity when compared to other roots and tuber crops, particularly cassava over the years was only marginal (Scott *et al.*, 2000; Asiedu and Sartie, 2010). Yam is a nutrient-loving plant generally referred to as "the first crop after bush fallow" due to its nutritional requirements (Carsky *et al.*, 2010). However, yam yield and productivity in response to soil fertility vary from one genotype to another (Matsumoto *et al.*, 2022). Consequently, soil fertility and nutrient management globally is a major concern in modern agriculture, with nitrogen as an essential nutrient, being the most limiting factor confronting the yield and productivity of many agricultural commodities, including yam (Franche *et al.*, 2009; Shiwachi *et al.*, 2015).

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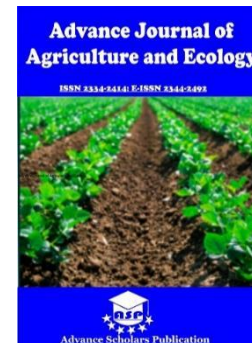


Nigeria, the world's largest yam producer, relies heavily on fertilizers (64 %) for its yam production (Carsky *et al.*, 2010), contributing approximately 67 % of the total world yam production (FAOSTAT, 2022), yam fertilization in Nigeria varies based on land-use history and cultural practices, thus resulting in recommendations ranging from 200 kg/ha to 600 kg/ha across yam geographical zones (Carsky *et al.*, 2010). Meanwhile, some yam varieties have previously been reported to thrive on low fertility, alkaline soil (Shiwachi *et al.*, 2015; Takada *et al.*, 2017). These studies indicated a positive association between these varieties of yam and some suspected plant growth-promoting rhizobacteria (PGPR), which are known for their ability to perform nitrogen fixation, indole acetic acid (IAA) production, phosphate solubilization and siderophores (Ouyabe *et al.*, 2019a, b). In the same vein, George *et al.* (2012) reported that nutrient uptake in yams depends on many factors, including plant root morphology, age, nutrient demand, supply, and utilization. Blanket fertilizer recommendations, however, have led to increased production costs in some yam-producing countries, with apparent limitations on soil fertility (Law-Ogbomo and Remison, 2008; Carsky *et al.*, 2010). Fertilizer recommendations in sustainable agriculture are therefore based on the soil nutrient status

through soil testing and analysis, previous crop history, particularly nutrient needs and estimated removal rates (Carsky *et al.*, 2010). Rezaei *et al.* (2017) reported that the dry weight of the aerial parts in treated plants was significantly higher compared to the control treatment, thus bolstering the claim of Suja (2001) who reported higher aboveground growth and enhanced chlorophyll content in white Guinea yam under higher N application rates. Both authors in their report observed that the application of N-based fertilizer delayed tuber initiation and prolonged the yam-growing season. Despite being the most cultivated and economically important yam species in West Africa, there have been no studies on its rhizosphere.

Cassava (*Manihot esculenta*), yam (*Dioscorea* spp.), and maize (*Zea mays*) are essential crops, providing a sizeable portion of the caloric intake for populations in Africa, Asia, and Latin America (FAO, 2019). Cassava is a drought-resistant root crop, often grown in poor soils, while yam is a tuber crop that plays a key role in many West African agricultural systems. Maize, a cereal crop, is grown globally and is critical for food, feed and biofuel production. Despite their importance, these crops are frequently grown in marginal soils with low fertility and are susceptible to diseases and pests. In this context, enhancing the microbial populations in the

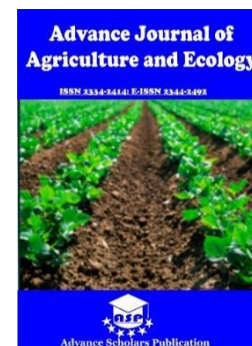
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rhizosphere could significantly improve the productivity, sustainability, and resilience of these crops (Gayan *et al.*, 2023). Cassava is one of the most important staple food crops in tropical regions and an understanding of the relationship between microbial communities and disease -resistance in cassava has remained elusive. Meanwhile, 16S rRNA and ITS of microbiome of ten cassava varieties analyzed, revealed a distinctive microbial community in the rhizosphere that showed significant interdependence with disease resistance. Shotgun metagenome sequencing unveiled the structure of microbiomes of cassava rhizosphere; the correlation between the rhizosphere microbiome and disease - resistance had been investigated (Zhang *et al.*, 2021). Cassava disease - resistance was significantly associated with *Lactococcus* sp., which specifically produces nisin, which has capacity to inhibiting the growth of *Xanthomonas axonopodis* pv. *manihotis* (Xam) and activating immune response in cassava. The new insights between cassava rhizosphere microbiome especially *Lactococcus* sp. and disease - resistance provide valuable information into further control of cassava disease (Zhang *et al.*, 2021). In cassava, yam and maize, the rhizosphere is critical for enhancing crop productivity by improving soil fertility and promoting plant health. Each crop's root system shapes its microbial community differently,

affecting how nutrients are cycled and how plants resist diseases. Cassava, for instance, is known for its association with nitrogen-fixing bacteria, which helps it thrive in nutrient-poor soils (Amir & Aslam, 2019). Maize, on the other hand, forms symbiotic relationships with arbuscular mycorrhizal (AM) fungi, which increase its phosphorus uptake (Smith & Smith, 2011), but for yam, microbial associations are less studied but are believed to involve fungi that aid in nutrient absorption and defense against pathogen attacks (Akanmu *et al.*, 2020). Recent research highlights showed that cassava roots are known to associate with nitrogen-fixing bacteria like *Azospirillum* sp. and *Azotobacter* sp., which help improve nitrogen availability in poor soils (Amir & Aslam, 2019). Yam studies have reported the presence of beneficial fungi such as *Trichoderma* sp. which protect the plant from root pathogens while also enhancing nutrient absorption (Akanmu *et al.*, 2020). A comparative study by Xu *et al.* (2017) on cassava and maize showed that while both crops harbor beneficial microbial populations in their rhizospheres, cassava tends to associate more with nitrogen-fixing bacteria, whereas maize showed stronger associations with phosphorus-solubilizing fungi. These differences highlight the crop-specific nature of rhizosphere microbial communities. Microorganisms in the rhizosphere can protect plants by outcompeting pathogens for resources,

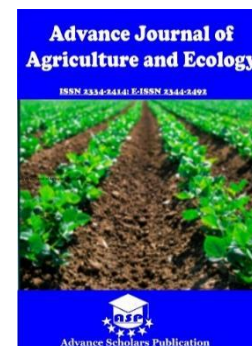
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producing antimicrobial compounds, or inducing systemic resistance in plants. In cassava, for instance, bacteria such as *Bacillus* and *Pseudomonas* have been shown to suppress *Phytophthora* sp., a major root pathogen (Ding *et al.*, 2024). Maize rhizospheres are often rich in biocontrol agents like *Trichoderma*, a fungus known for its ability to suppress soil-borne disease-agents such as *Fusarium* (Zhang *et al.*, 2019). Yam, though less studied, has been reported to benefit from the presence of *Trichoderma* and *Streptomyces* in its rhizosphere. These microorganisms not only suppress pathogens like *Sclerotium* and *Fusarium* but also promote healthy root development, leading to better yields (Marcia-Rodriguez *et al.*, 2020). Yam's microbial communities are less well-documented, but research suggests that yam-associated microbes, such as *Trichoderma* and *Penicillium* species, play important roles in decomposing organic matter and releasing essential nutrients like potassium and phosphorus (Olayinka *et al.*, 2018). These fungi also act as biocontrol agents, suppressing pathogens that attack yam tubers, such as *Sclerotium* and *Fusarium*, this biocontrol ability varies among cassava, yam and maize, depending on the microbial species that dominate each crop's rhizosphere (Akanmu *et al.*, 2020). Cassava is susceptible to several soil-borne pathogens, including *Phytophthora*,

which causes root rot. These bacteria also promote plant growth by stimulating root development and enhancing nutrient uptake, creating a healthier plant more resistant to disease. Maize is frequently affected by fungal pathogens like *Fusarium* and *Rhizoctonia*, which cause root rot and stalk rot. However, beneficial fungi like *Trichoderma* and *Gliocladium* species in the maize rhizosphere act as biocontrol agents by producing enzymes that break down the cell walls of pathogenic fungi (Zhang *et al.*, 2019). These biocontrol agents not only suppress pathogens but also promote root growth, improving overall plant health and productivity. Cassava (*Manihot esculenta*), yam (*Dioscorea* spp.), and maize (*Zea mays*) produce distinct root exudates, which serve as chemical signals attracting different microbial populations. Root exudates include sugars, amino acids, organic acids, and secondary metabolites, all of which play crucial roles in shaping microbial diversity. For instance, cassava's root exudates are rich in organic acids, which promote the growth of nitrogen-fixing bacteria like *Azospirillum* and *Rhizobium*, helping cassava thrive in low-nitrogen soils ((Nguyen *et al.*, 2016; Amir & Aslam, 2019). In contrast, yam releases exudates that are less studied, but research suggests they may favour beneficial fungi, such as *Trichoderma* species, known for their roles in enhancing nutrient

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uptake and suppressing pathogens (Akanmu *et al.*, 2020). Maize is highly dependent on mycorrhizal associations for nutrient uptake, particularly phosphorus. In addition, nitrogen-fixing bacteria such as *Rhizobium* and *Azospirillum* are commonly found in the maize rhizosphere, further contributing to nitrogen availability (Guo *et al.*, 2018). Maize has a fibrous root system that exudes sugars and amino acids that promote the colonization of arbuscular mycorrhizal fungi (AMF), especially *Glomus* species (Smith and Smith, 2011). These fungi form symbiotic relationships with maize roots, helping the plant access phosphorus, a nutrient often limiting in tropical soils (Guo *et al.*, 2018). Microbial communities in the rhizosphere play key roles in nutrient cycling, particularly nitrogen, phosphorus, and potassium, which are critical for plant growth. The objectives of the current study were to evaluate the microbial diversity in the rhizosphere of yam, maize and cassava as well as the impact of the environmental factors on the microbial communities in tropical soils southwest, Nigeria.

MATERIALS AND METHODS

This study was conducted with agricultural soil samples obtained from different farmlands southwest Nigeria at a depth of 0 - 30 cm; Atan (Ogun State), Ibadan (Oyo State), and Ojo (Lagos State). These areas were chosen due to their

different soil types and crop diversity, which provides a good background for comparing microbial communities in the rhizospheres of cassava, yam and maize.

History of sampled locations

Atan (Ogun State)

Atan is a semi-urban community located in Ogun State, southwest Nigeria. The area is known for its extensive agriculture, particularly in the cultivation of root crops such as cassava and yam. Atan has a tropical climate, with an average annual rainfall of about 1,200-1,500 mm and a temperature range of 25-30°C. (Balogun *et al.*, 2019).

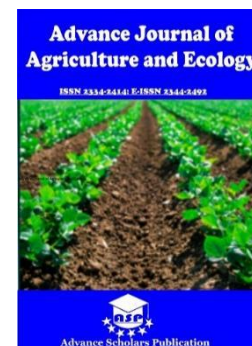
Ibadan (Oyo State)

Ibadan has a tropical rainforest climate, with an average annual rainfall of about 1,200-1,400 mm and temperatures ranging from 21-29°C. It has a long rainy season, making it ideal for the cultivation of maize, yam and cassava (Akanmu *et al.*, 2020)

Ojo (Lagos State)

Ojo is a growing peri-urban area in Lagos State, characterized by its humid tropical climate. The region has an average annual rainfall of 1,500-2,000 mm and temperatures ranging between 23-32°C. Soil from Ojo is clayey loam, which provides moderate water retention, making it suitable for crops like cassava and maize (Balogun *et al.*, 2019).

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The selection of Atan, Ibadan and Ojo as study areas for this research was based on their suitability for investigating microbial communities in the rhizospheres of cassava, yam and maize. These locations provide diverse agro-ecological zones and soil types, which are essential for understanding how environmental factors influence microbial communities and their interaction with crops.

SAMPLE COLLECTION

The procedure involved the random collection of soil from the rhizosphere. The collection dates were carefully planned to coincide with the critical growth phase to ensure the most accurate representation of microbial activity; the samples were collected during the peak vegetative growth stage of cassava, yam and maize (June). This stage was specifically chosen because the plants were actively growing, and root exudates (organic compounds secreted by roots) were expected to be most abundant. This sampling process was essential to capture the microbial communities that play a significant role in nutrient cycling, plant health, and productivity in cassava, yam and maize.

SAMPLING METHOD

A sterile soil auger was used to collect the soil samples. The auger was inserted into the soil at each selected point to a depth of 30 cm. The soil collected from each point was carefully transferred into sterile black plastic bags. For

each crop, cassava, yam and maize; five sampling points were identified around different plants in each study area. These points were strategically selected to reduce the impact of localized variations and to ensure that the microbial communities sampled were representative of the entire plot. The composite sample created from these five points provided a comprehensive overview of the microbial populations within the rhizosphere of each crop.

Control soil samples: control soil samples were obtained from Badagry beach at the same depth and transported to the laboratory, thereafter they were sterilized in the autoclave at 121°C for 15 minutes.

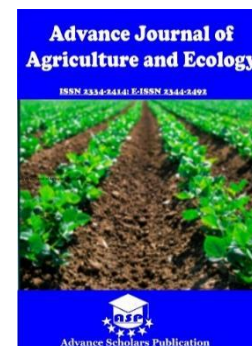
Sterilization and contamination control

All tools, including the soil auger and containers, were sterilized using 100 % ethanol before each use, this is in compliance with strict aseptic procedures as well as each soil sample was then stored in coolers at 4°C to preserve microbial viability during transport to the laboratory. Maintaining low temperatures during transport was crucial to prevent microbial degradation or activities before analysis (Smith *et al.*, 2020).

Microbiological analysis

Microbial populations in the rhizosphere were isolated using a combination of serial dilution and plating techniques. These methods allowed for the quantification and identification of both bacterial and fungal species present in the

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rhizosphere of cassava, yam and maize (Gerhardt *et al.*, 1981). Heterotrophic bacterial and fungal populations were evaluated with cultural technique using Nutrient agar (NA) and Sabouraud dextrose agar (SDA) respectively (Jones *et al.*, 2018). The Nitrogen-fixing soil bacteria were cultured on Yeast-extract mannitol salt agar (YEMA) supplemented with BTB (25µg/mL). The composition of Yeast Extract Mannitol agar; Mannitol (10g/L), K₂HPO₄ (5g/L), MgSO₄ .7H₂O (0.2g/L), NaCl (0.1g/L), Yeast Extract (0.5g/L), and Agar-agar (20g/L) (Ramakrishna, 2020). The salts were dissolved in 1000 ml of distilled water with mannitol and yeast extract added under continuous stirring. The pH was brought to about 6.8 with NaOH after which the agar-agar was added. Bromothymol blue (BTB) was prepared by adding 0.02g of bromothymol blue into 100 ml of distilled water. Then 10ml of the resulting solution was introduced into the YEMA medium. The medium was then autoclaved at 121°C for 15 minutes in order for it to be sterilized. The YEMA-BTB was prepared to aid in the differentiation between Fast-growing rhizobia (*Rhizobium*) producing an acid reaction in the YEMA medium (yellow colour) containing BTB (pH 6.8) while slow-growing rhizobia (*Bradyrhizobium*) produce an alkaline reaction (blue or purple colour) Thereafter, inoculated

plates were incubated at (26 ± 2°C) for 48 hours (Miller *et al.*, 2017).

Morphological examination of fungal cells

The potential fungal spore head, mycelium color, and hyphae characteristics were examined using the Microscope. Young mycelium was cut from the periphery of each culture with a sterile razor blade and placed on clean glass slides. Cut sections were stained with lacto-phenol cotton blue and examined under the microscope with the × 40 objective lenses (Smith, 1981; Cheesbrough, 2006). The bacterial cells were examined with the binocular microscope (Olympus, Japan) ×100 objective lenses

Biochemical characterization of microbial isolates

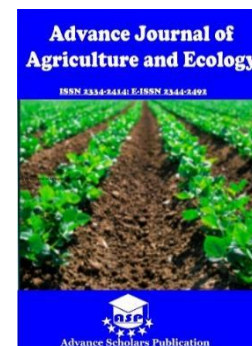
Biochemical characterization of microbial isolates was to confirm the identity of isolates using oxidase test, catalase test, motility test, starch hydrolysis, gelatin hydrolysis and gram staining reaction (Gerhardt *et al.*, 1981)

DNA extraction and amplification of the I6S rRNA gene

DNA extraction

A measurement of 80mg (wet weight) of bacterial cell that had been re-suspended in up to 200µl of deionized water to a ZR BashingBead™ Lysis Tube was obtained. It was secure in a bead

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beater fitted with a 2.0ml tube holder assembly (Scientific Industries' Disruptor Genie™, Cat. No. S6001-2 from Zymo Research Corp.) and processed at maximum speed for 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a micro-centrifuge at $\geq 10,000 \times g$ for 1 minute. Up to 400µl supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 rpm ($7,000 \times g$) for 1 minute. The base of the Zymo-Spin™ IV Spin Filter was snapped off prior to use. 1,200µl of Bacterial DNA Binding Buffer was added to the filtrate in the collection tube of step four. Thereafter, 800µl of the mixture from step five was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at $10,000 \times g$ for 1 minute. The flow through was discarded from the Collection Tube and step six was repeated. Then, 200µl DNA Per-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at $10,000 \times g$ for 1 minute. Thereafter, 500µl Fungal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at $10,000 \times g$ for 1 minute. The Zymo-Spin™ IIC Column was transferred to a clean 1.5ml micro-centrifuge tube and 100µl DNA Elution Buffer was added directly to the column matrix. It was then centrifuged at $10,000 \times g$ for 30 seconds to elute the DNA. The bacterial DNA were extracted following Zymo research

protocols (Guter & Koshland Jr. 1990; Kamusoko *et al.*, 2021; Qiagen, 2016).

PCR Amplification of the ITS/16S rRNA Gene and Sequencing

Polymerase chain reaction (PCR) was carried out to amplify the ITS gene of the fungal isolates using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction was carried out using the Solis Biodyne 5× HOT FIREPol Blend Master mix as described by Guter & Koshland Jr. 1990; Solanki, 2012 and Kamusoko *et al.*, 2021. The bacterial isolates 16S rRNA gene was amplified following the protocols of Qiagen (Qiagen, 2016).

16S rRNA / ITS Analysis:

PCR amplification of the 16S rRNA gene was performed using the following primers:

Forward Primer (with T7 tail):

TAATACGACTCACTATAGGGAGAGTTTGATCC
TGGCTCAG

Reverse Primer: GYTACCTTGTTACGACTT

PCR amplification of the ITS gene was performed using the following primers:

Forward primer (ITS1):

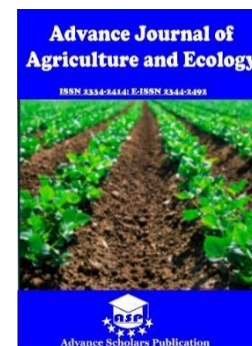
TCCGTAGGTGAACCTGCGG

Reverse primer (ITS4):

TCCTCCGCTTATTGATATGC

PCR is performed using the amplification program: 96°C (5 min), followed by 30 cycles of

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96°C (15 sec.), 52°C (15 sec.), 72°C (90 sec.). PCR completed with 5 mins. incubation at 72°C.

Approximately, 5µl aliquot from each PCR reaction was run on 1% TAE Agarose gel to confirm positive PCR outcome. PCR products were treated with ExoSAP and were sequenced (Sanger Sequencing) (Guter and Koshland Jr. 1990; Qiagen, 2016). The sequence information was analyzed using NCBI Blast. (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Analysis

In order to identify organisms, DNA sequence data generated from this study was blasted against

ITS/16S rRNA type strain database on NCBI. Threshold for identification was set according to Wang *et al.* (2014) with modification to accommodate species with high congeneric sequence divergence range. Phylogeny was constructed to support the identification by ITS sequence using Fast Minimum Evolution algorithm as deployed on NCBI and visualized on MEGA11 (Tamura *et al.*, 2021).

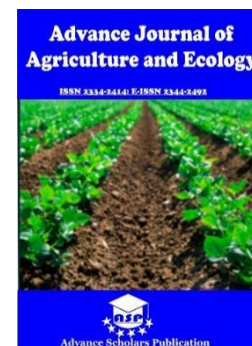
RESULTS

The differences in soil types across these locations was suggestive of variations in microbial composition and function. Atan's sandy loam, Ibadan's loamy soil, and Ojo's clayey loam each offer different conditions in terms of structure, nutrient availability, and moisture

retention. This allowed for a comparative analysis that underscores of how the edaphic factors shape microbial populations in the rhizosphere of the selected crops (Table 1).

These areas are known for their established agricultural practices. Each location has extensive cassava, yam and maize cultivation systems. Studying these practices provides fundamental basis for understanding how agricultural management impacts microbial interactions with plant roots, particularly in the rhizosphere. The microbial population dynamics incontrovertibly revealed the presence of more nitrogen-fixing bacteria (4.2×10^{-6} cfu/mL) at Atan than Ojo (3.8×10^{-6} cfu/mL) and Ibadan (3.0×10^{-6} cfu/mL), this suggested that the edaphic factors at Atan supported the proliferation of nitrogen-fixing bacteria (Table 2 and 3). Although, Atan had the least concentration of NH_4^+ and highest organic C concentration which enhanced the soil fertility (Table 1 and 2). Heterotrophic bacteria population were highest at Ojo (clayey loamy soil) while fungal population were least at the same location. However, fungal population was highest at Ibadan (Table 2) which suggested highest occurrence of Arbuscular Mycorrhiza Fungi (AMF), this was evident by the detection of highest PO_4^{3-} concentration at this location. It thus becomes incontrovertible that the nutrient cycling process and the microbial population

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dynamics formed significant factors that determined both the degree of soil fertility and crop yield. The observed differences in temperature, rainfall, and soil conditions between Atan, Ibadan, and Ojo offer valuable insights into how microbial communities adapt to diverse ecological stresses. This helps to understand the role of beneficial microbes in promoting plant growth and resilience under different environmental conditions (Fig. 2,3,4,5,6,7) (Gayan *et al.*, 2023).

The standard and conventional methods for microbiological analysis revealed through morphological and biochemical tests that

bacterial isolates were of different genera while the colonial and macroscopic characterization of the fungal isolates (Smith, 1981) showed that they belong to the genera; *Penicillium*, *Aspergillus*, *Fusarium*, and *Trichoderma* (Table 2 and 4). The different fungal populations in each of the agricultural fields were enormous and due to paucity of funds only a few were analysed following established standards to arrive at the identity of some of the isolates. This is suggestive of presence of Arbuscular mycorrhiza fungi (AMF) associations in the three investigated fields in varying concentrations (Fig. 1).

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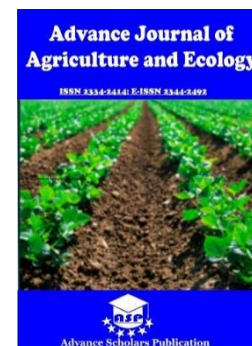


Table 1: Selected Physic-Chemical Analysis of composite Soil Samples

Parameter	Ojo	Atan	Ibadan SD		Mean
pH	6.69	6.88	6.53	6.70	0.175
NO ₃ , mg/L	23.73	20.24	19.41	21.13	2.292
NH ₄ , mg/L	8.20	6.69	7.99	7.63	0.818
PO ₄ , mg/L	1.83	2.31	2.62	2.25	0.398
TOC, %	2.91	3.10	2.73	2.91	0.185
Pb, mg/L	<0.001	<0.001	<0.001		0.001
Ca, mg/L	585.275	537.509	682.321		601.70
K, mg/L	160.646	161.309	73.790		149.88
Mg, mg/L	92.334	98.687	127.688		92.94
Na, mg/L	232.452	235.169	19.223		212.73
CEC, meq/100g	5.118	4.946	87.809	5.09	0.139
			5.465		
			170.555		
			36.546		

Key:

TOC= Total organic carbon

CEC= Cation exchange coefficient

SD= Standard Deviation

Table 2: Mean microbial populations in colony forming units (cfu/mL) on NA, SDA and YEMA

Sample	NA (cfu/mL)	SDA (cfu/mL)	YEMA (cfu/mL)
Atan	3.3×10^{-6}	3.5×10^{-6}	4.2×10^{-6}
Ojo	4.1×10^{-6}	3.2×10^{-6}	3.8×10^{-6}
Ibadan	3.5×10^{-6}	3.7×10^{-6}	3.0×10^{-6}
Control (Badagry beach sand)	0	0	0

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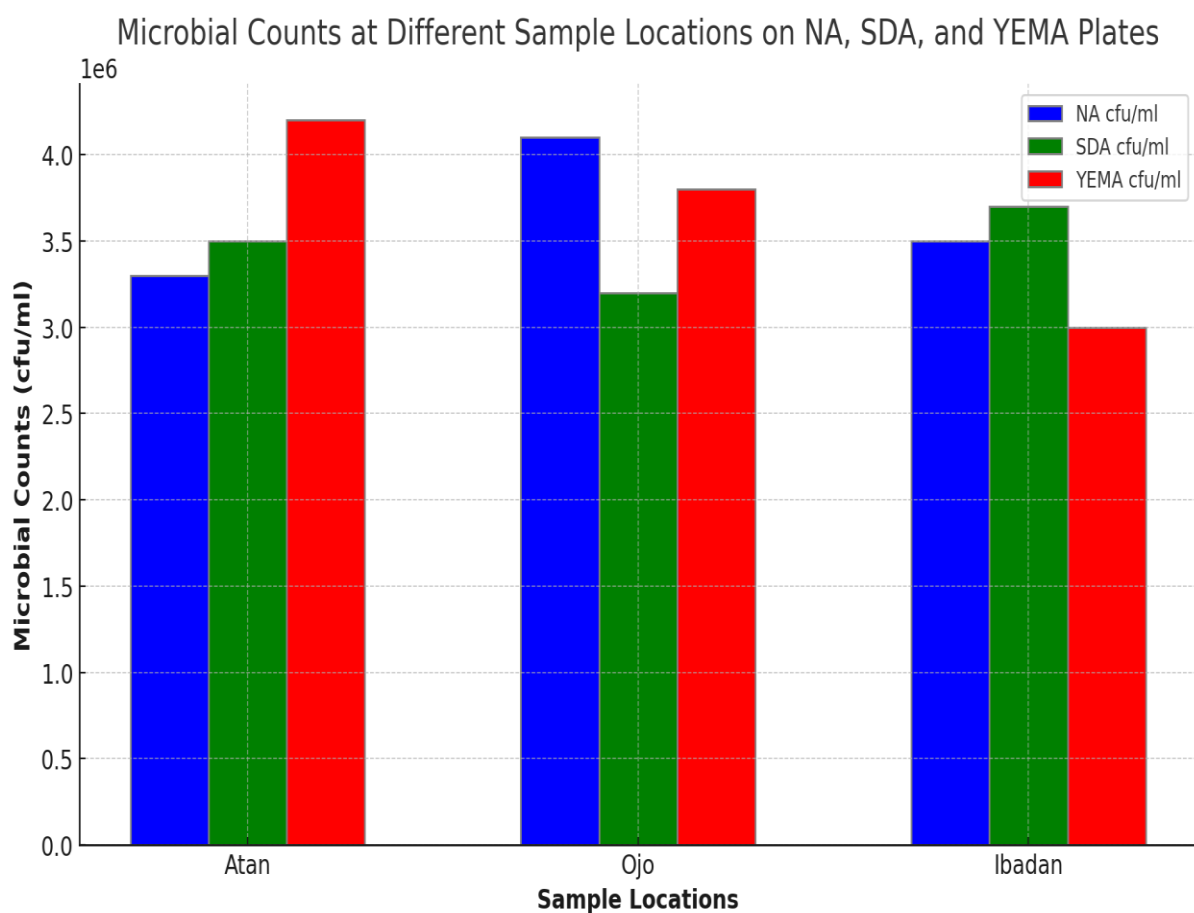


Figure 1: Microbial populations in the composite soil samples from locations

The bar chart illustrates the microbial counts (cfu/mL) on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), and Yeast Extract Mannitol Agar (YEMA) for soil samples collected from three different locations: Atan, Ojo, and Ibadan.

YEMA generally had the highest microbial counts, particularly at Atan, while NA had the highest count in Ojo. The variations in microbial populations between different agar media and locations highlight the influence of environmental factors on microbial growth across different regions.

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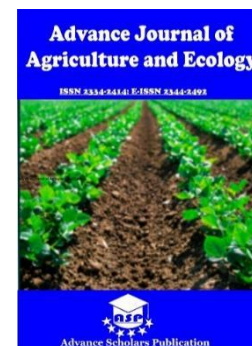


Table 3: Biochemical characterization of selected bacterial isolates on YEMA

Location	Gram reaction	Catalase test	Oxidase test	Motility test	Starch hydrolysis	Gelatin hydrolysis
Ojo	-	+	+	+	+	-
Ibadan	-	+	+	-	+	-
Atan	-	+	+	-	+	-

Key

+ = Positive

- = Negative

Table 4: Colonial and microscopic characteristics of rhizospheric fungal isolates

Fungal isolates	Macro-culture (colonial characteristics)	Microscopy (Morphological features)
<i>Aspergillus niger</i>	Black colonies	Non-septate conidiophores, Aerial hyphae
<i>Fusarium</i> sp.	white fluffy colonies	Branched conidiophores, non-septate hyphae
<i>Penicillium</i> sp.	Blue - green colonies	Branched conidiophores, septate hyphae
<i>Trichoderma harzianum</i>	White colonies	Branched conidiophores, septate hyphae

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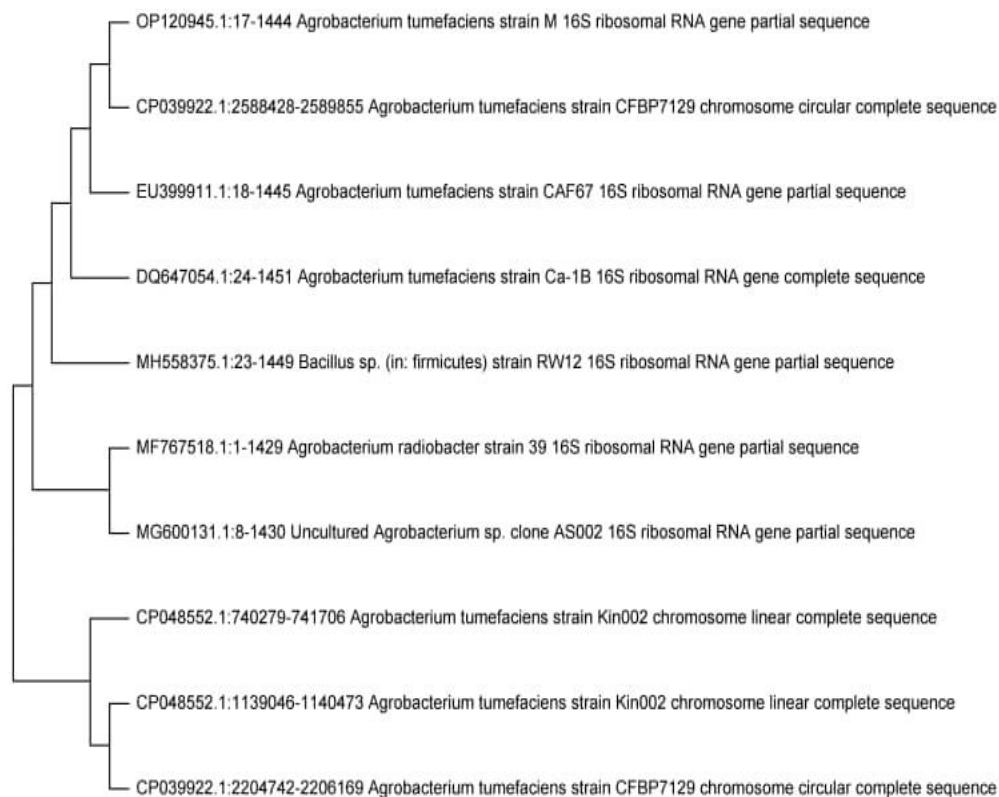
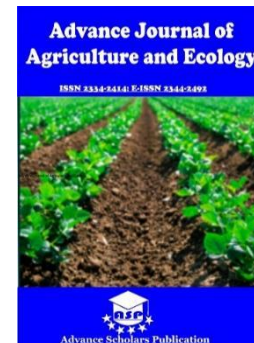


Fig. 2: Phylogeny tree of *Agrobacterium tumefaciens* from the plots of the three crops

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Fig. 3: Phylogeny tree of *Rhizobium rhizolycopersici* strain DBTS2 from rhizosphere of *Zea mays* and *Manihot esculenta*

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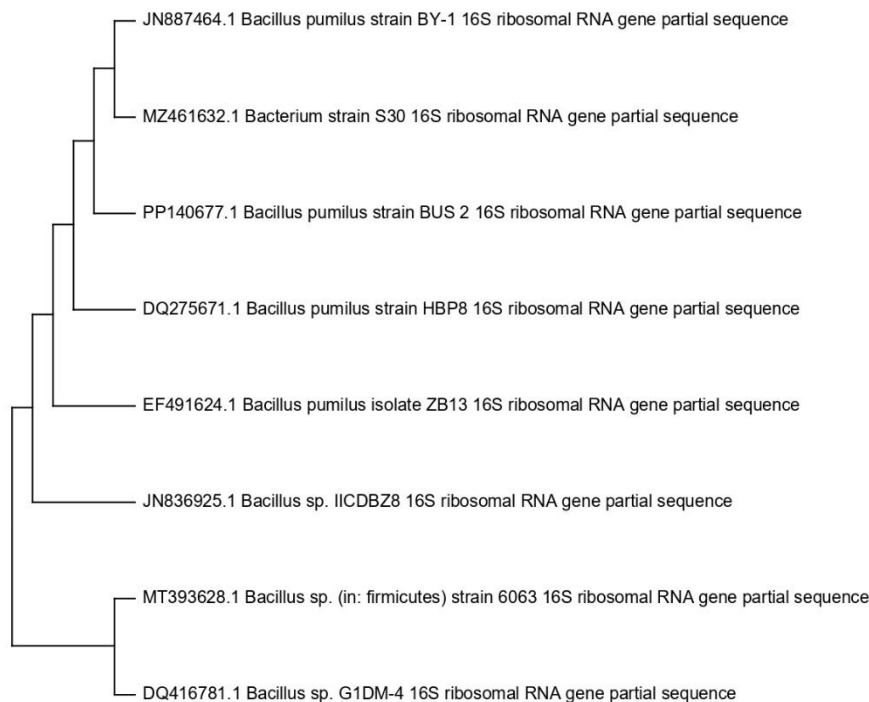


Fig. 4: Phylogeny tree of *Bacillus pumilus* from rhizosphere of the three crops

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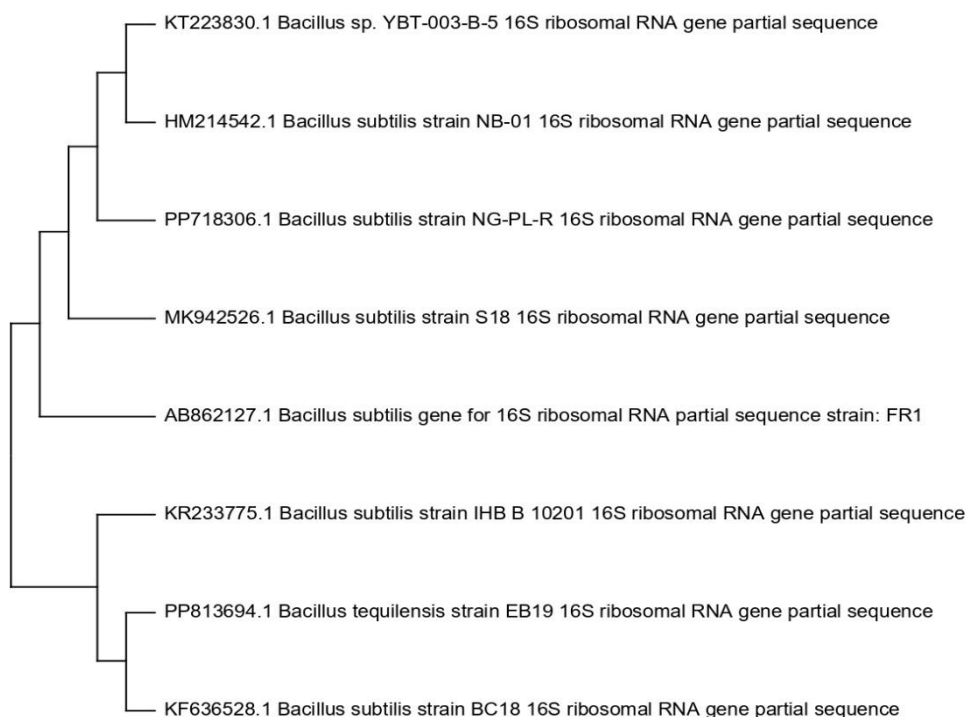
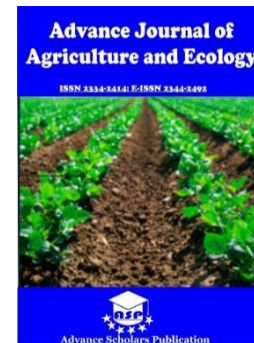


Fig. 5: Phylogeny tree of *Bacillus subtilis* from rhizosphere of *Zea mays* and *Dioscorea rotundata*

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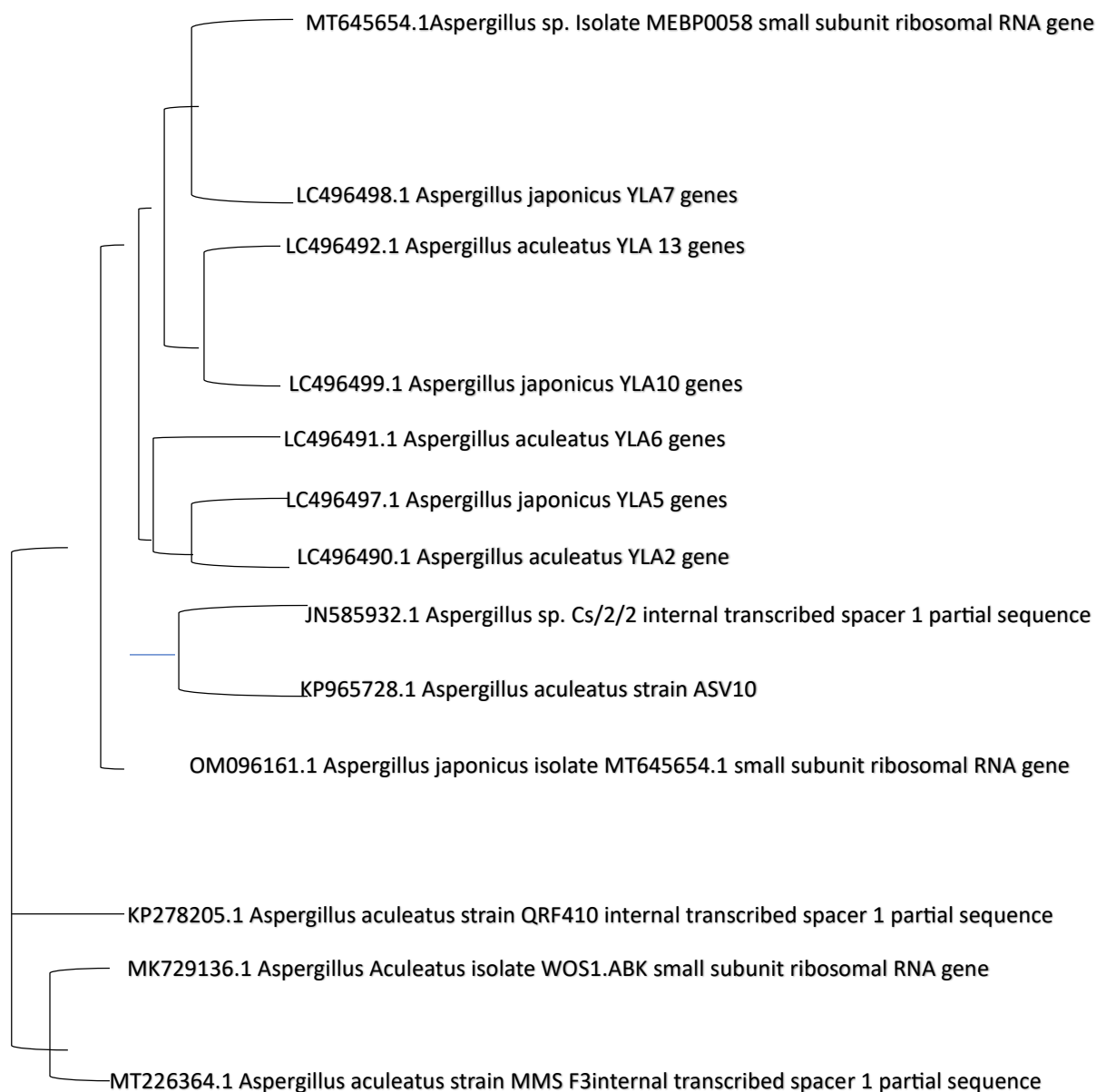
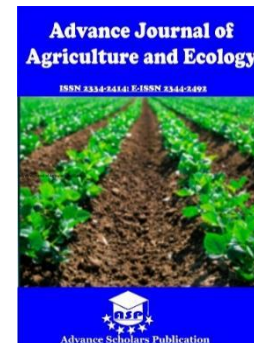


Fig.6 :Phylogeny of *Aspergillus japonicus* from yam and maize rhizospheres

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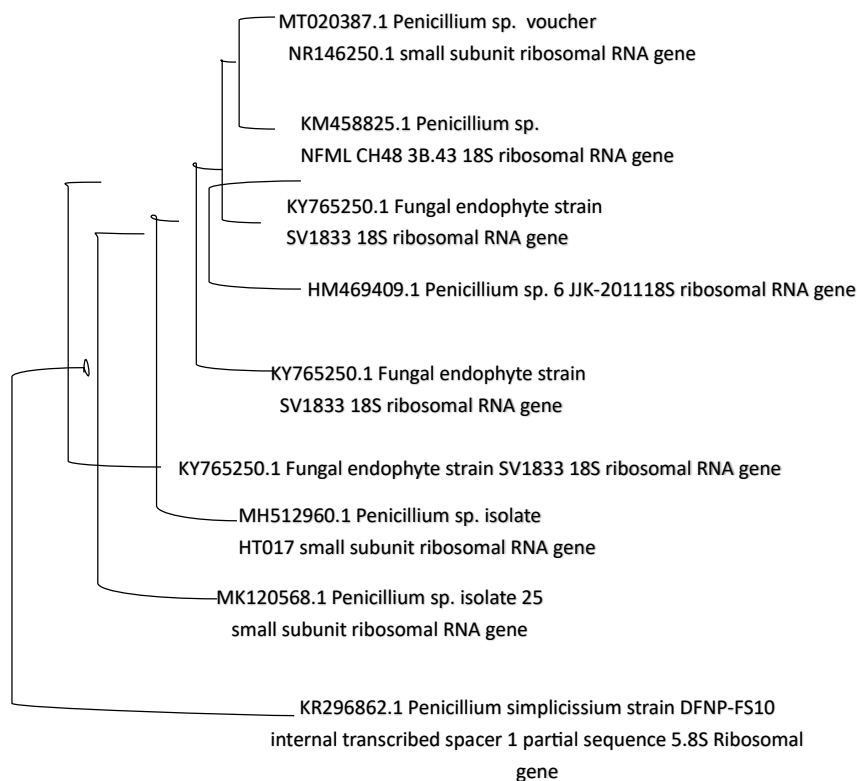
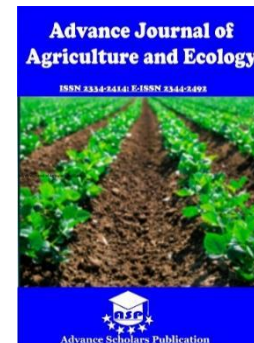
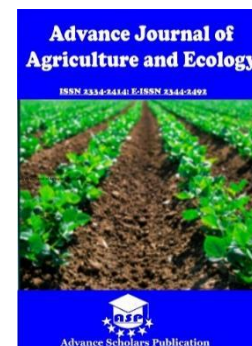


Fig.7: Phylogeny of *Penicillium simplicissium* from maize and yam rhizosphere

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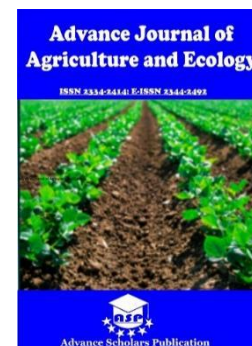


DISCUSSION

The rhizosphere is a chemically complex environment that serves as habitat for diverse microbial communities which, if exploited through strategic integration of effective microbial populations, crop management strategy and climate, could enhance crop yield and soil fertility. The discovered microbial communities in the rhizosphere of the three staple crops investigated were both complementary as well as capable of facilitating the reduction of various biotic and abiotic stress conditions through the metabolic products of the microbes and root exudates. This corroborated the findings of Lupwayi *et al.* (1998), Gayan *et al.* (2023), Chepsergon and Moleleki (2023). This study evaluated the microbial populations indigenous to the rhizosphere of three staple crops: yam (*Dioscorea sp.*), maize (*Zea mays*), and cassava (*Manihot esculenta*) cultivated in Southwest Nigeria. The microbial counts from three different locations; namely Atan, Ojo and Ibadan, revealed significant variations that reflect the influence of local soil characteristics and environmental conditions on microbial community dynamics. The presence of organic matter also promotes microbial diversity, particularly beneficial fungi like *Trichoderma sp.* and nitrogen-fixing bacteria (Zhang *et al.*, 2019; Marcia-Rodriguez *et al.*, 2020). Ibadan's fertile soil and favourable climate provide a unique

environment to study the microbial rhizospheres of maize and yam. The presence of organic-rich loamy soil supports a diverse range of soil microbes that play a crucial role in nutrient cycling, especially in maize, which relies heavily on microbial associations for phosphorus and nitrogen uptake (Akanmu *et al.*, 2020; Marcia-Rodriguez *et al.*, 2020). The clayey loam soil in Ojo is generally rich in minerals, but its structure can limit aeration, which may affect the composition of microbial communities. However, cassava thrives in such soils due to its deep root system, which interacts with microbes like nitrogen-fixing bacteria and phosphate-solubilizing fungi. The limited aeration in clayey loam soils may foster anaerobic microbes, adding a different dimension to the microbial dynamics (Zhang *et al.*, 2021). Soil from Ojo is peculiar due to its contrasting soil type compared to Atan and Ibadan. The slightly heavier soil can impact the microbial composition and activity in the rhizospheres of cassava and maize. The differences in microbial diversity and activity in Ojo, compared to the other locations, will provide a broader understanding of how soil structure affects plant-microbe interactions (Afolabi, 2020). The soil in Atan is predominantly sandy loam with moderate organic matter content, which is beneficial for crops like cassava that require well-drained soil. Sandy loam supports root development and

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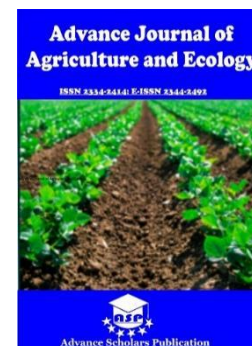
enhances the interactions between plant roots and beneficial microorganisms, such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria, which are crucial for cassava's growth in nutrient-poor soils (Zhang *et al.*, 2021). The importance of Atan lies in its extensive cassava and yam cultivation, which will allow for an in-depth study of microbial rhizospheres in a region where these crops are staples. Studying this area will provide insights into how soil type and agricultural practices in Atan influence microbial populations and how these microbes contribute to crop productivity (Afolabi, 2020).

In **Atan**, the heterotrophic microbial counts recorded were 3.3×10^6 cfu/mL on Nutrient Agar (NA), 3.5×10^6 cfu/mL on Sabouraud Dextrose Agar (SDA), and 4.2×10^6 cfu/mL on Yeast Extract Mannitol Agar (YEMA) supplemented with BTB. The higher count on YEMA+ BTB suggested a substantial presence of nitrogen-fixing bacteria, particularly *Rhizobium* species that are adapted to tropical acidophilic environment (Ojo-omoniyi *et al.*, 2015). *Rhizobium* species have been known for enhancing soil nitrogen levels and promoting plant growth (Murray and Thomas, 2002; Guo *et al.*, 2018). The balanced microbial populations across different media suggested that the environmental conditions at the three locations were conducive to both bacterial and fungal

growth, this is critical for nutrient cycling processes as well as mitigating environmental stress conditions and facilitating healthy plant growth. The detection of AMF suggested the occurrence of effective biogeochemical cycle and regulation of rhizosphere microbial community (Zhao *et al.*, 2023). The diversity observed aligns with previous findings that indicated healthy rhizosphere microbial communities which contributed to improved nutrient availability and overall plant health (Barea *et al.*, 2005; Zhang *et al.*, 2021; Zhao *et al.*, 2023).

The results from **Ojo** were noteworthy, as the heterotrophic bacterial counts were highest on NA at 4.1×10^6 cfu/mL, while SDA and YEMA recorded 3.2×10^6 cfu/mL and 3.8×10^6 cfu/mL respectively at this location. The predominance of heterotrophic bacterial populations suggested that the soil at Ojo was particularly conducive to bacterial growth, which played a crucial role in organic matter decomposition and nutrient cycling, consequently impacted on the degree of soil fertility at this location (Zhao *et al.*, 2023). The slightly lower fungal count suggested specific limitations within the fungal community which may be due to absence or presence of inadequate populations of Arbuscula Mycorrhizae fungi (AMF), which is essential for nutrient solubilization and soil health (Gorham *et al.*, 2013; Zhao *et al.*, 2023). The presence of ecological balance amongst the microbial

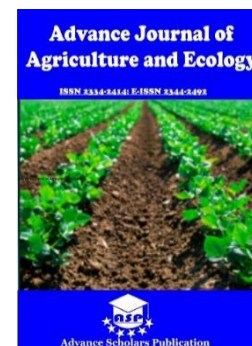
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communities in the rhizosphere is crucial for nutrient cycling, since the microbial populations performs complementary functions in soil ecosystems (De Vries *et al.*, 2018). In **Ibadan**, the microbial counts were relatively balanced, with 3.5×10^6 cfu/mL on NA, 3.7×10^6 cfu/mL on SDA, and 3.0×10^6 cfu/mL on YEMA. The relatively higher fungal counts suggested a diverse fungal community as well as adequate AMF presence that contributed to phosphorus solubilization (Table 1), which is vital for plant growth (Huang *et al.*, 2020). However, the lower count of nitrogen-fixing bacteria may limit the nitrogen availability in the rhizosphere, potentially affecting crop productivity. The variations in microbial populations across the three locations underline the importance of local edaphic factors in influencing microbial community dynamics. Factors such as soil texture, pH, and organic matter content significantly affect microbial activity and diversity, for instance, the soil in Ojo, with its favorable physicochemical properties, supported a higher total heterotrophic bacterial count, reflecting its capacity for nutrient retention and microbial activity. These findings correlate with previous studies that suggested that soil structure impacts nutrient uptake by plants and microbial community composition (Govaerts *et al.*, 2009; Zhang *et al.*, 2021). Furthermore, the overall microbial diversity observed in this study

further substantiated the importance of ecosystem resilience, enabling soils to respond to environmental changes and stresses (Van der Heijden *et al.*, 2008; Gayan *et al.*, 2023). The presence of diverse microbial communities in the rhizospheres is crucial for enhancing ecosystem services such as nutrient cycling, disease suppression, and soil fertility, this corroborated the submission of Chepsergon and Moleleki (2023). The presence of beneficial microbes, such as nitrogen-fixing bacteria and plant growth-promoting rhizobacteria (PGPR), underscores the potential for these microorganisms to improve soil fertility, plant-growth promotion and crop resilience (Ouyabe *et al.*, 2019b; Gayan *et al.*, 2023). The functional roles of microbes in the rhizospheres of cassava, yam and maize differ, reflecting the distinct nutritional demands of these crops. In addition, cassava's rhizosphere is known to harbor phosphate-solubilizing bacteria, which help release phosphorus from insoluble compounds in the soil (Johnson *et al.*, 2015; Zhang *et al.*, 2021). Yam rhizosphere microbial communities are less well-documented, but research findings suggested that yam-associated microbes, such as *Trichoderma* and *Penicillium* species, play important roles in decomposing organic matter and releasing essential nutrients like potassium and phosphorus (Olayinka *et al.*, 2018). These fungi also act as biocontrol agents, suppressing

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pathogens that attack yam tubers, such as *Sclerotium* and *Fusarium* (Akanmu *et al.*, 2020). This is in agreement with the findings in this particular study where *T. harzianum* and *Penicillium* species were isolated from yam and maize rhizospheres. Therefore, understanding the interactions between specific microbial populations and their effects on plant health can lead to better management practices that supports sustainable agriculture. This study underscores the significance of microbial populations in the rhizospheres of yam, maize and cassava as well as the need for synergistic interactions between soil structure, microbial community and edaphic factors. The findings reveal critical insights into the roles that these microbial populations play in enhancing nutrient uptake, soil health, and crop productivity in southwest Nigeria. Farmers can reliably capitalize on indigenous microbial populations, climate and edaphic factors to implement sustainable agricultural practices that impacts positively on crop yields and maintain soil fertility. Future research should explore the interactions between specific microbial populations and their effects on crop health as well as soil fertility, further elucidating the potential for microbial interventions in agricultural systems.

CONCLUSION

The evaluation of microbial populations in the rhizosphere of yam (*Dioscorea spp.*), maize (*Zea mays*), and cassava (*Manihot esculenta*) in southwest Nigeria highlights the significant role that soil microorganisms play in enhancing plant growth, nutrient uptake, and disease resistance. The implications of these findings are far-reaching for sustainable agricultural practice in Nigeria and sub-Saharan Africa. By promoting beneficial microbial communities through practices such as organic amendments and the use of biofertilizers, farmers can enhance soil health and reduce reliance on chemical or synthetic fertilizers, thereby minimizing adverse environmental impacts. The microbial communities make vital influences on ecosystem resilience against adverse climatic changes as well as reduce greenhouse gas emissions while simultaneously mitigating various biotic and abiotic stress conditions that pose increasing challenge to sustainable agriculture. There is the need to emphasize climate smart agriculture mediated by microbial communities in soil for sustainable agriculture to achieve its aims. Future research should focus on the specific interactions between crops and their associated microbial communities, exploring innovative practices that harness these relationships to boost crop productivity and enhance food security especially in developing countries.

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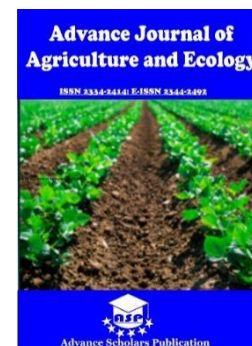
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Author Contributions: The study was conceptualized, designed and preparations by Ojo-Omoniyi, O. A., Olanbiwoninu, A. A. and Fashogbon, R. while data collections, analysis by Travih, S. I. and editing by Ahuekwe, E. F., Oziegbe, O., and Moro, D.D. and All authors contributed equally in the editing of the manuscript. All authors read and approved the final manuscript.

Dataset: All Dataset are available in GenBank and NCBI repository, the Accession numbers for isolates are displayed and trackable. (www.ncbi.nlm.nih.gov/genome).

Ethical Consent: Ethical approval is not required for this type of work in Nigeria, since we did not test any material on human and animal subjects.

Consent to Participate: The consent to participate in this study was given by each author.

Consent to Publish: The consent of each author was obtained prior to the commencement of the publishing process

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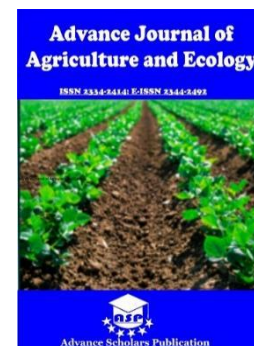
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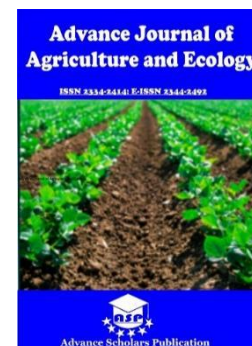
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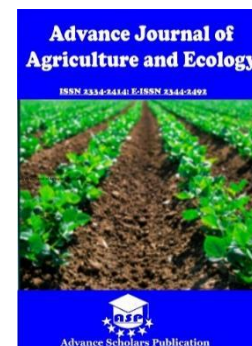
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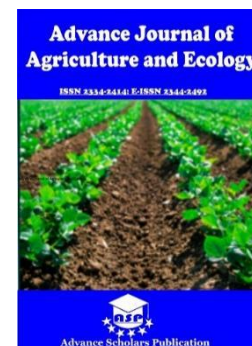
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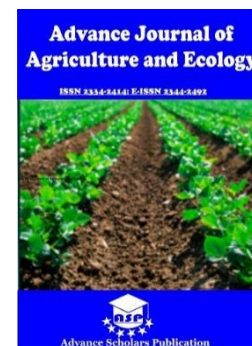
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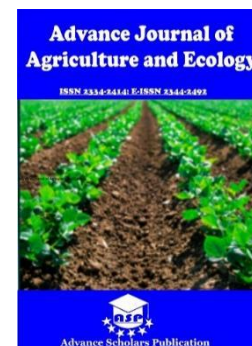
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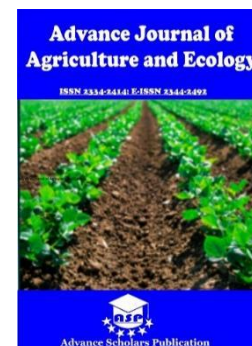
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