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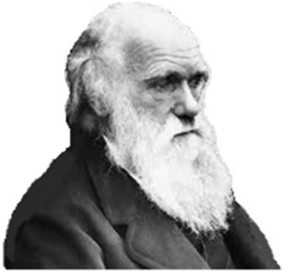

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Examining the Effects of Biofertilizer Microbes on Plant Growth and Nutrient Dynamics in the Rice Rhizosphere in Ebonyi State, Nigeria

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ABSTRACT

In Ebonyi State, Nigeria, this study delves at the isolation and characterization of biofertilizer microorganisms connected to the rice rhizosphere in a variety of agroecological zones. The goal of the study is to clarify how these microbial communities promote nutrient uptake and solubilize vital minerals to improve plant growth. In a greenhouse, four rice accessions (Faro 62, Faro 61, Faro 52, and Faro 59) were grown alongside control samples (unplanted soil). Throughout the course of several growth stages, a thorough examination of the microbial community structure and its relationship to rice growth factors was carried out. The results indicate a significant relationship between microbial diversity and plant health, suggesting that specific microbial strains can potentially be harnessed to improve sustainable agricultural practices.

***Keywords*:** Biofertilizer, Microbial communities, Rice rhizosphere, Plant growth promotion, Nutrient uptake, Soil health, Microbial diversity

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1. INTRODUCTION

The variety of bacterial populations in the rice rhizosphere under two distinct management approaches; conventional tillage and no-tillage is examined by [1]. The researchers looked at the bacterial diversity at different phases of rice growth using a culture-based methodology. The findings highlight how crucial soil management techniques are in forming microbial communities, which may have consequences for sustainable agriculture. The study [2] examines the effects of several slow-release fertilizer application techniques on the variety of microorganisms in the rice rhizosphere. The study highlights the benefits of mechanical deep fertilization over conventional broadcasting techniques for raising agricultural output and microbial diversity. The biodegradability of agro-biopolymer-based slow-release or controlled-release fertilizers (SRF/CRF) is investigated by [3]. It draws attention to the benefits of employing biopolymers, which enhance soil quality and nutrient release efficiency due to their renewability, biodegradability, and low environmental risk.

The study comes to the conclusion that although adding biopolymer can accelerate biodegradation, this effect is not always favorable and is dependent on the SRF/CRF composites' physicochemical characteristics. Careful planning is required to balance the effects on the environment and nutrient release due to the intricacy of the biodegradation pathways. In Northeast China, gross primary production (GPP), grain production, and paddy rice farming were recorded notable increases between 2000 and 2017 [4]. Technological developments and changes in agricultural policy are blamed for this expansion, which has increased photosynthetic activity and productivity. In light of environmental changes, the authors emphases on the significance of these trends for food security and sustainable agriculture techniques.

The study [5] examines the effects of different slow- and controlled-release fertilizers on rice yield. It compares the efficacy of various fertilizer types and emphasizes how they affect crop growth and overall yield performance. Findings show that some combinations greatly increase rice yield, indicating that choosing the right kind of fertilizer helps maximize agricultural production and sustainability. The microbiome of the rice rhizosphere is less variable and has a higher species richness than bulk soil [6]. It emphasizes how interkingdom interactions between bacteria and archaea have increased and how rice roots stabilize microbial ecosystems. Furthermore, genes involved in the metabolism of carbon, sulfur, and iron are abundant in the rhizosphere, offering information that may support sustainable farming methods in paddy fields. By comparing planted soils with unplanted controls, the greenhouse experiment could provide insights into how different rice accessions influence microbial diversity and stability, similar to the findings related to the homeostatic effects of rice roots in the original study [6].

This connection emphasizes the role of specific rice varieties in shaping rhizosphere microbiomes, which can impact nutrient cycling and overall plant health. The isolation and characterization of plant growth-promoting rhizobacteria (PGPR) from Dentami, a local pea variety that is produced organically in Sikkim, India [7]. The research emphasizes how these bacteria can improve plant growth by fixing nitrogen, solubilizing phosphate, and producing compounds that stimulate plant growth. The results advance our knowledge of the advantageous interactions between PGPR and plants, which can guide the development of sustainable farming methods and raise crop yields. The various ways that free-living rhizospheric bacteria can promote plant growth and Numerous bacterial strains that can promote plant growth through processes including nitrogen fixation, phosphate solubilization, and phytohormone synthesis [8], which results in highlighting the potential for increasing crop output and advancing sustainable agriculture practices through the use of particular rhizospheric bacteria as biofertilizers. The focuses on the identification and isolation of thermophilic bacterial strains from the Central Himalayan Region of India's Soldhar hot spring, Eleven different strains of high-temperature-loving bacteria were found by the researchers [9]; these strains grow at pH values of 5 to 10 and temperatures between 20 and 100 °C. The genetic resemblance of two strains to Geobacillus thermocatenulatus and Geobacillus thermoleovorans was noteworthy, indicating the versatility and possible biotechnological uses of thermophilic bacteria in harsh conditions. The examination of how microbial communities in arable soil are affected by eight years of treatments with both organic and inorganic fertilizers [2, 10].

According to the study, the makeup of bacterial and fungal communities is greatly influenced by the combination of organic manure and inorganic fertilizers. In comparison to other treatments, the OMIF (organic manure-inorganic fertilizers) treatment notably displayed increased bacterial richness but reduced fungal richness. Furthermore, changes in the nitrate level and organic matter of the soil were associated with differences in the abundance of particular bacterial and fungal phyla. These results demonstrate how different microbial populations react to prolonged fertilization techniques. The study [11] investigates how PGPR, or plant growth-promoting rhizobacteria, can improve crop resilience and productivity. The authors go over a number of ways that PGPR can boost plant growth, including boosting disease resistance, increasing stress tolerance, and improving nutrient uptake. The potential of PGPR applications in sustainable agriculture is emphasized in the review, especially in light of the growing food demand and climate change. In order to increase agricultural output, the study emphasizes the need of utilizing advantageous microbial interactions by showcasing new developments and useful applications.

The focus of the study [12] is on the identification and isolation of rhizobacteria from soil, investigating their potential as microbes that stimulate plant development and emphasizes how root exudates, which provide bacteria with nutrients, impact the rhizosphere, which is a crucial area of microbial activity. After isolating rhizobacteria, the scientists evaluated how well they could solubilize phosphate and generate indole-3-acetic acid (IAA). Investigations into the effects of these plant growth-promoting rhizobacteria (PGPR) on the development of black-eyed pea (chawali) and matki (mat) seeds revealed their potential advantages in agricultural techniques. The bacterial populations linked to several rice cultivars in flooded soils in Ebonyi State, Nigeria, are examined in the paper [13]. The study examines the physicochemical characteristics of soil and the concentrations of pore water ions in various rice fields using molecular methods. The presence of both inorganic and organic materials, as well as the identification of major soil types and somewhat acidic pH levels, are important discoveries. The researchers used RAPD PCR with certain primers to identify a variety of polymorphic alleles among the bacterial isolates.

Notable bacteria found include *Escherichia coli*, *Aeromonas hydrophila*, and *Pseudomonas plecoglossicida*; the bulk of these bacteria are classified as gamma proteobacteria. Understanding of microbial interactions in agricultural ecosystems is improved by the study's insightful analysis of the structure of the bacterial population in rice paddy soils. Pseudomonas kribbensis and Burkholderia cenocepacia were among the eight native rhizobacterial strains that [14] isolated and described from the rhizosphere of organic rice fields in Sikkim, India.

Plant growth indices like root length, leaf number, grain yield, and biomass were all markedly increased by these strains. They also exhibited antagonistic activity against phytopathogens that impact big cardamom, with Consortia-3 showing the strongest growth-promoting effects for rice. According to [5, 15], rice plants' rhizodeposition increases microbial activity in the rhizosphere when compared to control or bulk soil. This could be harmful to the growth of some specific bacteria while favoring the growth of others. [2] explored how microbial communities in arable soil under a rice-wheat system were affected by eight years of organic manure-inorganic fertilizers (OMIF) and inorganic fertilizers (IF). The results indicated that while bacterial diversity reduced with IF treatment, fungal richness fell and bacterial richness increased with OMIF treatment. The results show a correlation between soil organic matter and nitrate levels and the varied effects of long-term fertilization strategies on fungal and bacterial communities. [16] use a new multi-channel microelectrode that is intended to monitor ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) simultaneously in order to study nitrification and denitrification processes in the rice rhizosphere. Their results show that whereas denitrification happens in the nearby anoxic soil, nitrification happens in the oxic surface layer. In planted rice microcosms, nitrification and denitrification were inhibited by the roots' substantial reduction of available nitrogen levels due to nitrogen assimilation; however, nitrification activity rebounded after the addition of nitrogen sources such as urea, helped by oxygen released from the rice roots.

The study [17] explore how salt and alkalinity affect ammonia-oxidizing microorganisms in the rhizosphere of saline-alkali tolerant rice planted in coastal solonchaks and discovered that this particular environment increased nitrification processes, which resulted in notable modifications to the ammonia-oxidizing bacteria's community structure. The work sheds light on the ways in which salt and alkalinity as well as two environmental stresses, can affect microbial interactions and the cycling of nutrients in rice rhizospheres. This information is useful in understanding the ecological dynamics of coastal agricultural systems. The examination of how bacterial community structures in bulk soil and the rhizosphere of maize are affected by artificial root exudates [18] , these exudates drastically alter the diversity and makeup of bacterial communities, favoring some while inhibiting others. With its emphasis on the significance of root exudates in the interactions between plants and soil as well as the cycling of nutrients, this study sheds light on how plants influence their microbial habitats.

The study of how temperature and increased CO_2 affect the dynamics of carbon (C) and nitrogen (N), as well as the emissions of nitrous oxide (N_2O) and methane (CH_4) in tropical flooded rice (*Oryza sativa* L) [19], rising temperatures and CO_2 have a major impact on soil C- and N- levels, which changes greenhouse gas emissions. These findings draw attention to the possible effects of climate change on rice production and the ensuing environmental consequences. The different phases of plant growth affect the rhizosphere microbiome's composition in Arabidopsis [20]. They discovered that, in contrast to subsequent developmental stages, unique microbial communities originate during the seedling stage, even while the general organization of the bacterial community stays unchanged and emphasizes the function of root exudates in favor of particular microbial populations, implying that plants actively mold their rhizosphere microbiome for diverse purposes as they grow.

The crucial role of rhizosphere microbes in enhancing plant stress tolerance, emphasizing their symbiotic relationships with plants [11, 21], which highlight that these microbes can improve nutrient uptake and promote resilience against environmental stresses, thereby contributing to overall plant health and productivity. In wheat crop fields, [22] examine the notable distinctions in the spatial distribution and bacterial network structure between rhizosphere soils and bulk soils. According to their research, the rhizosphere is home to a more intricately linked microbial population that is essential to soil health and plant health. According to [23], the microbial co-occurrence pattern in the wheat rhizosphere is less complex and more stable than in the bulk soil.

The microbial community in the rhizosphere may be more robust, which could improve soil stability and plant health. The vital role, rhizosphere bacteria play in improving plant stress tolerance, [24] highlight the symbiotic interactions that these microbes have with plants and emphasize how these microorganisms might enhance nutrient uptake and foster resistance to environmental stressors by enhancing the general health and productivity of plants. These studies [22,23,24] show that these microbial communities are important for plant health and productivity in agricultural environments. They do this by improving plant tolerance to stress, displaying unique spatial structures, or exhibiting stability. When compared to unplanted soil, [25] investigate the dynamics of microbial communities in the rice rhizosphere over four growth stages reveals that rice cultivation increases microbial enzyme activity and biomass. Their findings show that bacterial and fungal community structures have undergone notable alterations, underscoring the critical function that rhizosphere microbes play in maintaining soil health and rice plant growth over time.

The concept of rhizosphere priming effects (RPE) in maize, revealing that root-mediated changes significantly enhance soil organic matter decomposition, especially in nitrogen-deficient conditions were explored by [26] and find that nitrogen availability can reduce RPE by lowering root exudation and microbial activity, despite an increase in root biomass. In investigation of the geographical distribution of enzyme activity in the rhizosphere, [27] show that the patterns are strongly influenced by the diameter of the roots and the root hairs. According to their research, the microbial enzyme activity in the surrounding soil is increased by finer root structures.

This suggests that root morphology is important for soil health and nutrient cycling. A thorough review of nitrogen cycling in the rhizosphere is given by [28], who highlight the critical role that plants play in shaping microbial populations and nitrogen transformations. Plant characteristics, such as root structure and exudate makeup, they contend, have a major impact on nitrogen availability and cycling dynamics, which in turn affects soil health and ecosystem function. The study by [29] reveals that the stage of soil development significantly influences the composition and diversity of bacterial communities in the rhizosphere of the pioneer plant Saxifraga oppositifolia along a High Arctic glacier chronosequence. The rhizosphere microbial community of the rare and endangered Lauraceae species Cinnamomum migao is shaped by both plant recruitment and bulk soil microbial reservoirs. The bulk soil bacteria are largely responsible for community formation [30]. In investigation of how interactions between plants and microbes within the rhizosphere microbiome improve resource acquisition, [31] emphasize the significance of root exudates in forming microbial communities that sustain plant health and productivity.

The diversity and antagonistic characteristics of Pseudomonas species in the rhizosphere of maize grown on a subtropical organic farm are examined by [32]. Their research highlights the importance of Pseudomonas species in sustainable agriculture by revealing a wide range of species that show potential biocontrol effects against plant infections. Higher pH levels in the rice rhizosphere microbiome improve nutrient cycling activities and increase the complexity and stability of co-occurrence networks, [33] research emphasizes the importance of soil pH in determining how microbes interact with one another and how ecosystems function in agricultural settings. The study [34] explores the role that functional features play in the rhizosphere's bacterial community selection, and it finds that plant interactions with soil microorganisms are species-specific rather than random. It implies that functional features are essential for the assembly of these microbial communities and emphasizes the importance of both taxonomic and functional diversity in forming them. [35] investigate the microbiological mechanisms and coupling processes that are engaged in the biogeochemical cycles of important elements in the paddy-rice rhizosphere and sheds light on how microbial activity affects soil health and nutrient cycling, offering suggestions for sustainable rice farming methods.

Three Acidobacteria genomes are analyzed by [36] in order to learn more about the ecological roles and lifestyles of these microbes in soil environments. Their varied metabolic capacities and adaptations that support their survival and function in a range of soil habitats are revealed by the study. The dispersal limitations and environmental selection shape bacterial and fungal communities in the broomcorn millet rhizosphere in Northern China were explored by [37] and their findings indicate that while fungal β-diversity is influenced by both factors, bacterial β-diversity is predominantly driven by environmental conditions, underscoring the complexity of microbial interactions in agricultural ecosystems. [37, 38] compare the rhizosphere to bulk soils in rice fields to examine the stability and adaptability of bacterial communities and discovered that rhizosphere populations are highly stable but show minimal environmental adaptation. They underscore the significance of the rhizosphere for crop productivity and soil health by indicating that its special circumstances lead to a more uniform microbial community structure. [39] investigated how agricultural management and plant selection together influence rhizosphere microbial communities and nitrogen cycling processes, revealing that these factors interact to shape microbial diversity and composition in maize agroecosystems.

The combined effects of plant selection and agricultural management on rhizosphere microbial communities and nitrogen cycling processes examined by [39].which showed that these variables interact to determine the microbial composition and diversity in maize agroecosystems. In order to improve plant health and the sustainability of ecosystems, the study emphasizes the significance of taking management strategies and plant selection into account. As emphasized by [20], the critical role that microbial communities play in fostering plant health and production by examining the formation of rhizosphere microbiomes and their notable effects on plant growth.

The study demonstrates how particular microbial interactions within the rhizosphere can affect plant development and improve nutrient availability. The use of phospholipid fatty acid (PLFA) analysis to examine the stability of the microbial population in the rhizosphere of Spartina alterniflora in both natural and altered environmental settings by [40]. Despite considerable fluctuation in abiotic variables, the results show minor changes in the composition of microbial communities, suggesting a high degree of resilience in the rhizosphere microbiota. The study [41] investigate how root exudation mechanisms in conservation agriculture affect the rhizoplane microbiota's consumption of components produced from microbes and exudates boost nutrient cycling and microbial interactions, which benefits plant development and soil health.

This study aims to isolate and characterize native rhizobacterial strains from the rhizosphere of rice in organic fields in Ebonyi state, Nigeria. It evaluates the plant growth-promoting effects of these strains on rice, focusing on parameters like root length, leaf number, grain yield, and biomass, while also investigating their antagonistic activity against crop phytopathogens for integrated pest management. Additionally, the research involves creating bacterial consortia to enhance plant growth and applying statistical techniques, such as PCA, to analyze the microbial community composition's relationship to plant growth outcomes.

2. METHODS

Using a soil auger, soil sample opaque polythene bags were filled with soil from the plow layer (0–20 cm) of rice fields located in the three agroecological zones of Ebonyi State: Ebonyi North, Ebonyi Central, and Ebonyi South. Ebonyi State is located in the Southern part of Nigeria. The geographical coordinate of Ebonyi State is 6°15’N 8°05’E. In Experimental Design: Rice seeds were germinated and transplanted into pots. Each accession was represented in three pots, with some pots left unplanted as controls. Soil properties were analyzed using AOAC standard methods for Physicochemical Analysis and Colony counting (cfu/ml) and 16S rRNA gene amplification for Microbial Characterization. Use of three RAPD primers (OPB05, OPT05, OPB03) for molecular characterization. In Plant Growth Measurement: Rice plant heights were measured at four growth stages (early vegetative to maturity). Statistical Analysis: Data were analyzed using one-way and two-way ANOVA with a significance level of 0.05.

The study employed a combination of field sampling, laboratory analysis, and statistical techniques to investigate the relationships between soil properties and rice growth parameters. This involved collecting soil samples from the study area, analyzing the samples for various physicochemical properties, and measuring the corresponding rice growth parameters, such as plant height. we then generate a correlation heatmap to explore the strength and direction of the relationships between the soil properties and rice growth indicators. Additional analyses were also conducted which are; quality filtering and processing of 16S rRNA sequence data to characterize the soil microbial community composition, and performing principal component analysis (PCA) to understand the underlying patterns and relationships in the microbial community data. Furthermore, we integrated the soil property and rice growth data with the microbial community analysis to gain a comprehensive understanding of the soil-plant-microbe interactions.

3. RESULTS AND DISCUSSION

3. 1. Soil pH Variation Across Agroecological Zones

Three distinct agroecological zones (labeled as Zone 1, Zone 2, and Zone 3) are displayed in Figure 1 below which shows the soil pH levels. With a pH range centered around 6.9, Zone 2 has the highest soil pH and the most mildly alkaline soil conditions. Every agroecological zone has a different pH of soil; the most acidic soils are found in Zone 3, the most neutral soils are found in Zone 1, and the somewhat alkaline soils are found in Zone 2.

Understanding the appropriateness of these zones for growing different crops is crucial because different crops do better in different pH ranges. Farmers and agricultural planners can improve productivity in each agroecological zone by selecting crops, applying soil amendments, and implementing management strategies based on a better understanding of the pH profiles of the soil.

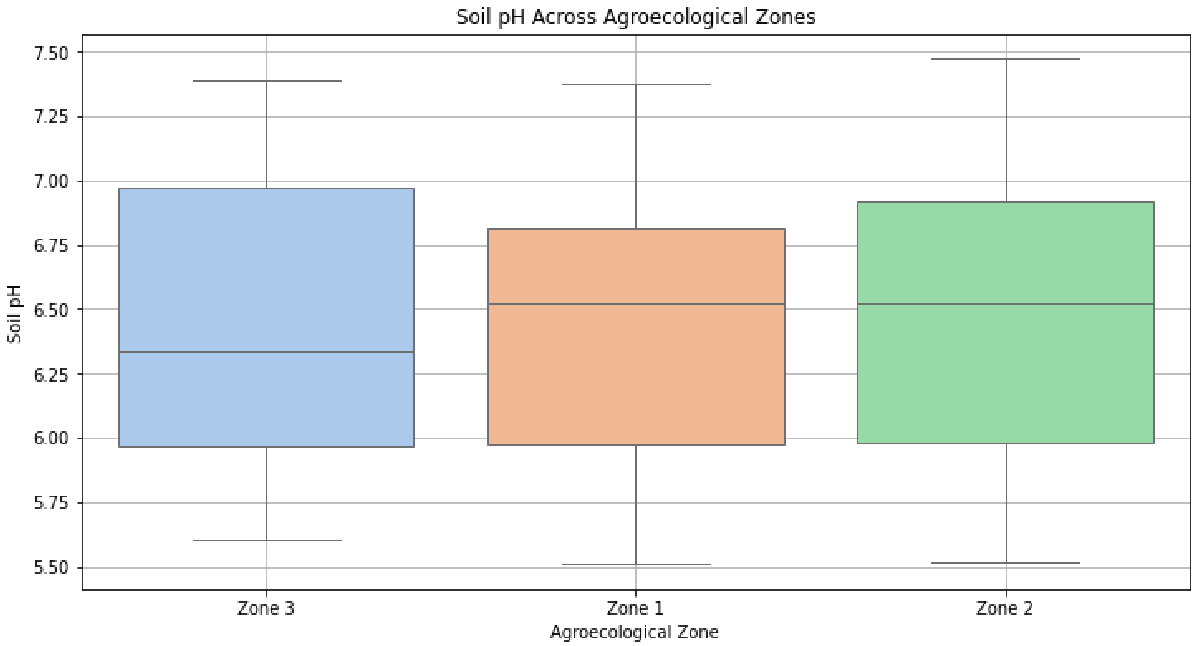

Fig. 1. The soil pH levels across three different agroecological zones.

3. 2. Exploring the Relationship between Microbial Load and Rice Height

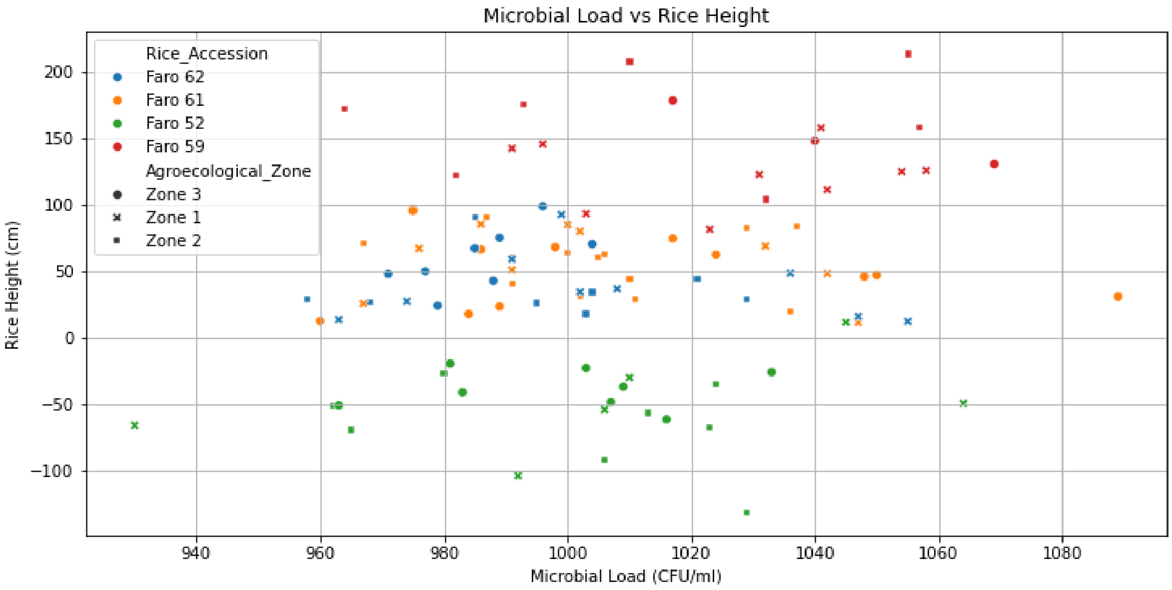

Fig. 2. Correlation between microbial load (measured in CFU/ml) and rice height (measured in cm).

The correlation between microbial load (measured in CFU/ml) and rice height (measured in cm) for various rice accessions and agroecological zones is depicted in the scatter plot in

Figure 2 below. The data points show a complex, non-linear pattern, indicating that factors other than the microbial community could additionally have an impact on the relationship between microbial load and rice height. The large range of rice heights (-50 cm to 200 cm) and microbial loads (940 to 1080 CFU/ml) suggests a wide variety of samples and growing environments. The data points' clustering according to agroecological zones and rice accessions indicates that both genetic and environmental factors are important in deciding how a plant will exhibit growth. The dispersed distribution of the data points suggests a complex, non-linear relationship between rice height and microbial load, where the observed patterns are probably influenced by a variety of factors, including the composition of particular microbial communities, interactions between plants and microbes, and environmental factors. The complex relationship between the microbial population and rice development is highlighted by this study, indicating that the underlying complexity may be too deep for a straightforward linear correlation to fully reflect. The important microbial species, their functions, and the precise processes by which they interact with the rice plant to affect growth and development must all be further investigated. Gaining an understanding of these intricate relationships might help with the development of focused strategies that maximize plant performance in various agroecological settings.

3. 3. Variation in Rice Height across Different Accessions

The data on rice height for four distinct rice accessions; Faro 62, Faro 61, Faro 52, and Faro 59, were displayed in the graph in Figure 3 below. The variations in rice height between these accessions are shown graphically in the graph. The tallest rice fields are Faro 62 and Faro 61, with median heights of roughly 60 to 70 cm and a restricted range of variance. With readings ranging from about -20 cm to 20 cm, Faro 52 shows a broader range of rice heights, suggesting greater variability within this accession. With a median height of approximately 160 cm and a greater range of variance than the other accessions, Faro 59 has the highest average rice height.

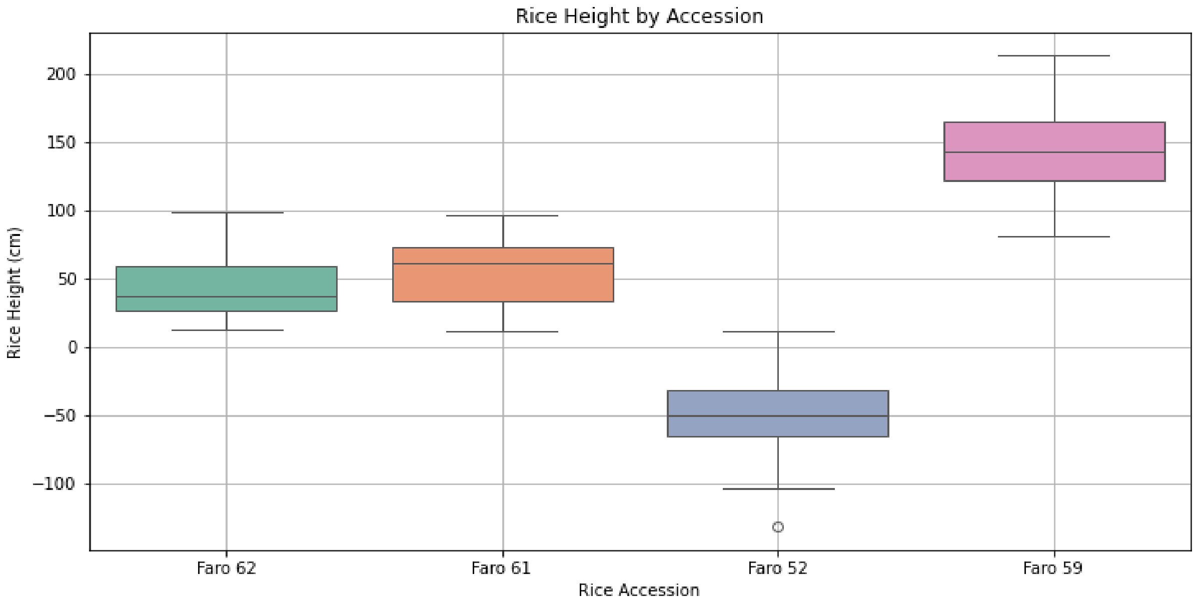

Fig. 3. The variations in rice height between these accessions

The observed variations in rice height across the four accessions point to a major role for genetics in dictating plant growth and development. The observed variances in rice height are the result of each accession's distinct traits and adaptation to the local environmental conditions. By choosing the best rice varieties for certain growing conditions with the aid of breeders and farmers, an understanding of these genetic variations can ultimately lead to increased agricultural output and sustainability.

3. 4. Correlation Matrix of Soil Properties and Rice Growth Parameters

A correlation heatmap that examines the connections between different soil characteristics and rice development parameters is shown in Figure 4 below. The intensity and direction of the relationships are shown visually in the heatmap.

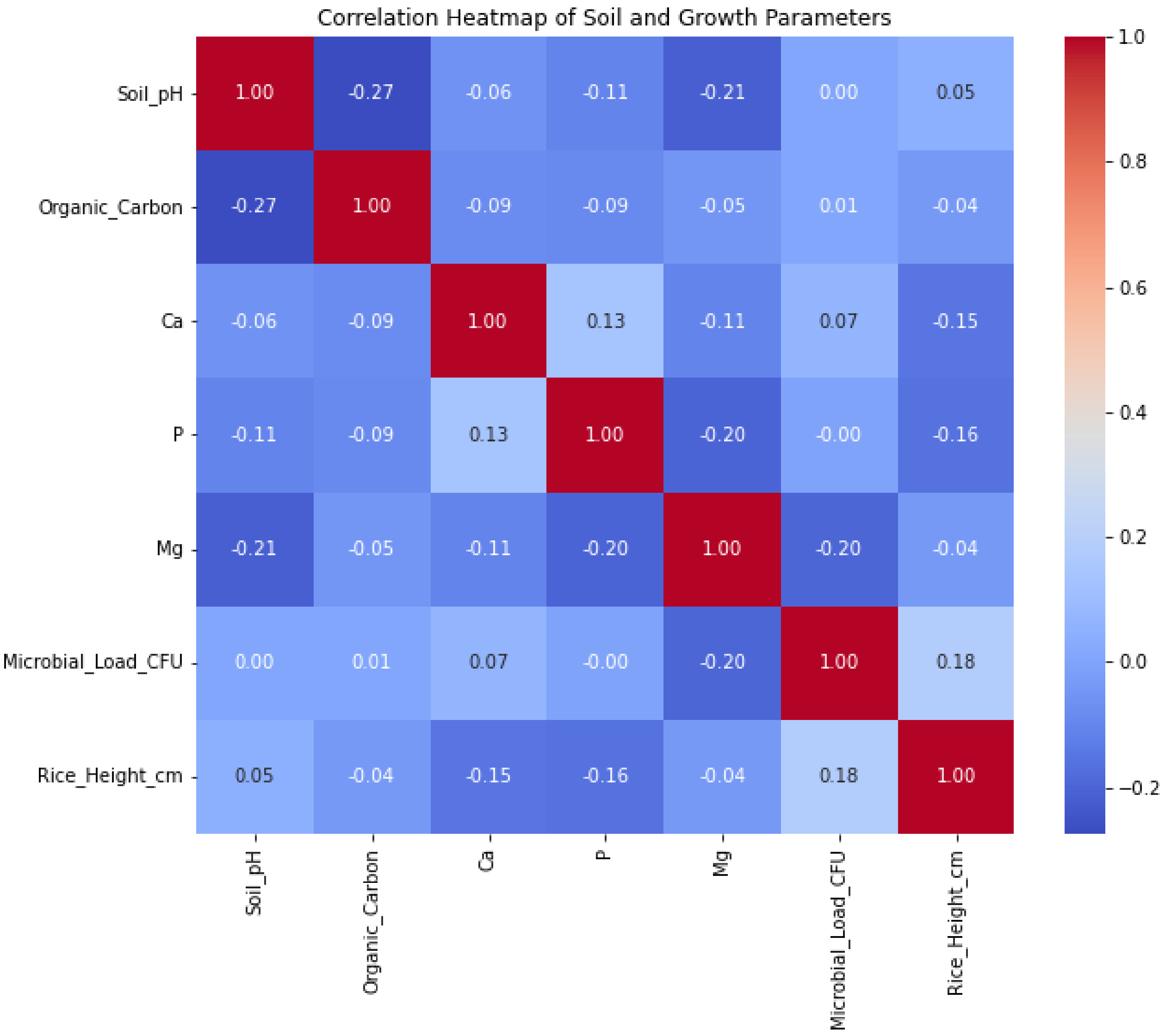

Fig. 4. Correlation heatmap of soil and growth parameters

There is a clear association between soil pH and other parameters with itself, as demonstrated by the strong positive correlation (1.00) that soil pH displays with other parameters and itself.

The organic carbon content may decrease as soil pH rises, according to the relative negative correlation (-0.27) between organic carbon and soil pH. The relative positive correlation (0.13) between calcium (Ca) and phosphorus (P) suggests that these two soil minerals may work in concert. The relative negative correlation (-0.20) between magnesium (Mg) and phosphorus suggests an antagonistic relationship between these two soil components. A weak positive correlation (0.18) between rice height and microbial load (CFU) suggests that the microbial community may have some, if restricted influence upon plant growth.

The correlation heatmap offers insightful information on the intricate connections between rice development factors and soil characteristics. These insights can help select suitable rice cultivars and direct soil management techniques to maximize agricultural productivity. The comparatively weak connections found, however, imply that additional elements, such genetics and environmental variables, might also be important in regulating rice growth and development.

The diagonal line formed by values of 1 in the correlation heatmap represents the perfect, positive correlation between each parameter and itself. The presence of these diagonal 1 values helps confirm that the correlation matrix is calculated correctly, as the self-correlation of any variable should always be 1. This diagonal line of 1s serves as a visual reference to validate the internal consistency of the correlation heatmap.

4. CONCLUSION

The results of this study illustrate the potential of biofertilizer bacteria to improve nutrient dynamics and plant growth, underscoring their crucial function in the rice rhizosphere. Rice growth metrics were found to be highly influenced by the various differences in microbial communities across different agroecological zones, highlighting the significance of choosing the right microbial strains for certain environmental conditions.

This study advances our knowledge of the relationships between soil, plants, and microbes and offers insightful information for creating sustainable rice-growing techniques. Future research should concentrate on applying these microbes in field settings to further evaluate the efficacy of identified beneficial bacteria in enhancing rice productivity and soil health.

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