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INFLUENCE OF NPK NANO FERTILIZER ON MICROBIAL COMMUNITIES AND CHEMICAL PROPERTIES IN PADDY RICE (*ORYZA SATIVA L.*)

[1]A. A. OLUBODE, [2]N. O. ADEYEMI, [2]E. O. ONI, [3]O. T. H. HAMZAT-AKINWANDE, [4]C. T. SENJOBI, AND [1]E. A. ODUDU

[1]Department of Soil Science and Land Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Department of Plant Production and Crop Physiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[3]Crop Research Programme, Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[4]Plant Science Department, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
***Corresponding Author**: adejoy212@gmail.com **Tel:** +2348036530946

ABSTRACT

Rice (*Oryza sativa* L.) production in sub-Saharan Africa faces challenges from declining soil fertility to inefficient nutrient management. This screen house study assessed the effects of banana peel-synthesized NPK Nano fertilizer at four rates (Control, 50%, 75%, and 100%) on soil microbial communities, enzyme activities, arbuscular mycorrhizal fungi (AMF) diversity and root colonization, nutrient availability, and root morphology in two lowland rice varieties (NERICA L-19 and WITA-4) using a 4 x 2 factorial in a Completely Randomized Design with three replicates. NPK Nano fertilizer significantly increased bacterial populations up to 9.9 x 10^5 CFU g^{-1}, fungal populations up to 19.0 x 10^3 CFU g^{-1}, and soil enzyme activities, including amylase (315.0–601.4 mg kg^{-1}), urease (21.80–24.90 mg kg^{-1}), and alkaline phosphatase (122.03–138.6 mg kg^{-1}). AMF root colonization, including arbuscules, hyphae, and vesicles, increased significantly in a dose-dependent manner, with 100% Nano priming reaching 74.33%, 49.50%, and 30.00%, respectively. Five AMF genera were identified: *Acaulospora, Funneliformis, Gigaspora, Glomus, and Rhizophagus*, with spore density inversely related to Nano fertilizer rate. Soil nutrient availability showed no significant treatment effects, likely reflecting efficient plant-mycorrhizal nutrient uptake. Root fresh weight was significantly highest under 100% Nano priming (42.5 g), while NERICA L-19 exhibited greater root elongation. PCA and correlation analyses confirmed strong positive relationships among Nano fertilizer rate, enzyme activities and AMF colonization. These findings establish NPK Nano fertilizer as a promising tool for enhancing the rhizosphere and promoting sustainable lowland rice production in West Africa.

Keywords: NPK Nano fertilizer; arbuscular mycorrhizal fungi; soil enzyme activity; banana peel extract; green synthesis; paddy rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary staple food for more than 3.5 billion people globally. It is a key source of food security in sub-Saharan Africa, where demand continues to outpace domestic production (Kumar *et al.,* 2023). Nigeria, the largest rice consumer and importer in Africa, faces a widening yield gap driven by soil nutrient depletion, low fertilizer use efficiency, and degradation of the soil biological environment on which crop productivity fundamentally depends (Sahoo *et al.,* 2024; Vasuki *et al.,* 2024). Conventional NPK fertilizer management, while providing macronutrients, often disrupts the delicate rhizosphere ecology by suppressing beneficial microbial communities, reducing enzymatic nutrient cycling, and diminishing the plant-mycorrhizal symbiosis central to nutrient acquisition efficiency in rice (Kalwani *et al.,* 2022).

Nanotechnology has emerged as a paradigm -shifting approach in precision agriculture, offering the potential for targeted, slow-release nutrient delivery via nano-sized carriers that markedly improve nutrient use efficiency, minimize leaching losses, and reduce environmental contamination (Yadav *et al.,* 2023; Singh *et al.,* 2024). Nano fertilizers, characterized by particle sizes of 1–100 nm and extraordinarily high surface-area-to-volume ratios, exhibit enhanced reactivity and solubility, increasing nutrient availability in the rhizosphere compared with conventional formulations (Liu and Lal, 2015; Bansal *et al.,* 2025). Specifically, green-synthesized NPK Nano fertilizers have shown promising results for rice productivity and soil biological quality in recent greenhouse and field experiments (Sahoo *et al.,* 2024; Thanta *et al.,* 2025).

Green synthesis of nanoparticles using plant-derived extracts has gained global recognition as a sustainable, cost-effective, and environmentally benign alternative to physico-chemical nanoparticle synthesis methods (Goh *et al.,* 2023). Banana (*Musa acuminata*) peels, an abundant agricultural by-product rich in reducing sugars, polyphenols, flavonoids, and pectin, have been successfully used as capping and reducing agents in diverse nanoparticle syntheses (Aswathi *et al.,* 2023). The valorization of banana peel waste for Nano fertilizer production aligns with circular bioeconomy principles and offers a locally accessible, low-cost Nano agronomic input for smallholder farmers in West Africa (Bishnoi *et al.,* 2023).

Soil microbial communities, particularly bacteria and fungi in the rhizosphere, constitute the biological engine driving nutrient cycling, organic matter decomposition, and soil structure formation (Gou *et al.,* 2023). Soil enzyme activities, including amylase (starch hydrolysis), urease (nitrogen mineralization), and phosphatases (phosphorus cycling), serve as sensitive indicators of soil biological quality and are closely linked to microbial community structure and diversity (Tang *et al.,* 2024). Arbuscular mycorrhizal fungi (AMF) form obligate mutualistic symbioses with the roots of approximately 80% of vascular plant species, including rice, thereby enhancing phosphorus uptake, drought tolerance, disease resistance, and root architecture (Guigard *et al.,* 2023; Xu *et al.,* 2024). The interplay among Nano fertilizer application, microbial ecology, enzymatic activity, and AMF symbiosis in the paddy rhizosphere remains poorly characterized, particularly for improved West African rice varieties.

Despite growing evidence of the agronomic

benefits of Nano-fertilizers, integrated assessments that concurrently evaluate effects on microbial populations, enzyme activities, AMF community structure, root morphology, and nutrient availability in tropical rice systems remain scarce. Elucidating these integrated biological responses is essential for developing evidence-based nano-fertilizer management protocols adapted to sub-Saharan African lowland rice production environments. This study was designed to: determine the effects of graded NPK Nano-fertilizer application on soil bacterial and fungal populations; determine impacts on soil enzyme activities; characterize AMF genus-level diversity, spore density and root colonization; determine soil nutrient availability and root morphological responses; establish multivariate relationships among measured parameters using PCA, Pearson correlation, and dose-response analyses.

MATERIALS AND METHODS

Experimental Site and Soil Preparation

The experiment was conducted in 2024 at the screen house of the College of Plant Science and Crop Production (COLPLANT), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria (latitude 7°15'N, longitude 3°25'E; altitude ~75 m a.s.l.). The study site is located within the tropical rainforest-derived savanna transition zone, characterized by bimodal rainfall (mean annual rainfall: 1,200–1,400 mm), ambient temperatures ranging from 25 to 33°C, and relative humidity of 75–85%. Soil samples for the experiment were collected from the FADAMA lowland area at 0–20 cm depths, air-dried under shade to constant weight and thoroughly homogenized by mixing. Eight (8) kilograms of the prepared soil were packed into 10 kg-capacity polyethylene buckets, which served as individual experimental units.

Experimental Design and Treatments

The experiment was laid out in a 4 × 2 factorial arrangement in a Completely Randomized Design (CRD), replicated three times (n = 24 experimental units). Factor A comprised four NPK Nano fertilizer application rates: T1 = Control (no NPK Nano fertilizer), T2 = 50% NPK Nano fertilizer, T3 = 75% NPK Nano fertilizer and T4 = 100% NPK Nano fertilizer. Factor B comprised two lowland rice varieties: V1 = NERICA L-19 (New Rice for Africa) and V2 = WITA-4 (West Africa and Indian varieties Timor). These varieties were selected based on their widespread adoption and agronomic importance in West African lowland rice production systems.

Preparation of NPK Nano fertilizer Using Banana Peel Extract

Uncontaminated, ripe banana (*Musa acuminata*) peels were collected, thoroughly washed with distilled water to remove surface impurities, and dried at 65°C in a forced-draft oven to constant weight. The dried peels were cut into small pieces and crushed with a mortar and pestle to increase the surface area available for bioactive compound extraction. The extraction process involved boiling 1 kg of crushed peel material in 5 litres of distilled water at 100°C for 2 hours under reflux conditions. Following extraction, the solvent–peel mixture was vacuum-filtered through Whatman No. 1 filter paper to remove particulate residues. The resulting liquid extract (Nanosizer) was stored at 4°C until use. NPK nano fertilizer was prepared by dissolving pre-calculated quantities of compound 0.95 g/L of NPK (15:15:15) fertilizer into the banana peel nanosizer solution at a mixing ratio of 1:5 (NPK Nanosizer, w/v). The Nano-formulated fertilizer was incorporated

into the experimental soil at the respective application rates before transplanting.

Rice Varieties and Transplanting

Seeds of NERICA L-19 and WITA-4 were surface-sterilized with 0.1% sodium hypochlorite for 5 minutes, rinsed with distilled water, and pre-germinated on moist filter paper. Fourteen-day-old uniform seedlings were transplanted at 2 seedlings per experimental bucket at 15 kg of soil per bucket. Routine irrigation was applied to maintain field capacity. No additional fertilizer was applied beyond the experimental nano-NPK treatments (0.95 g/L of NPK (15:15:15) fertilizer into the banana peel nanosizer solution at a mixing ratio of 1:5 (NPK nanosizer, w/v).

Microbial Population Enumeration

Rhizospheric soil samples were collected aseptically at 10 weeks after transplanting (10 WAT) using a sterile sampling device. Bacterial populations were determined by the standard serial dilution pour-plate method on Nutrient Agar (NA; Oxoid), with incubation at 28°C for 24–48 hours. Colonies were counted and expressed as CFU g^{-1} oven-dry soil. Fungal populations were quantified on Potato Dextrose Agar (PDA; HiMedia) supplemented with 100 µg mL^{-1} streptomycin sulfate, with incubation at 25° C for 5–7 days in the dark. Fungal colonies were expressed as CFU g^{-1} dry soil.

Soil Enzyme Assays

Soil enzyme activities were determined from freshly collected rhizosphere soil samples within 24 hours of collection. Amylase activity was measured by incubating 1 g of soil with 0.5% starch solution at 37°C for 1 hour, and quantifying the released glucose colorimetrically. Protease activity was determined by the casein hydrolysis method, urease by the phenol-indophenol colorimetric method (Kandeler and Gerber, 1988), and sulphatase by the p-nitrophenol release method (Tabatabai and Bremner, 1969). Acid phosphatase (ACPA), alkaline phosphatase (ALPA), and neutral phosphatase (NEPA) were measured by the p-nitrophenyl phosphate method at the appropriate pH (pH 6.5, 11.0, and 7.0, respectively). All enzyme activities were expressed as mg substrate kg^{-1} dry soil h^{-1}.

AMF Spore Counting and Species Identification

Rhizospheric soil (0–15 cm depth) was randomly sampled from each pot at 10 WAT. Three 20 g soil subsamples were wet-sieved through a 45–710 µm sieve series using a method modified from Gerdemann and Nicolson (1963) to ensure efficient spore recovery from the fine-textured paddy soil. The sieved fraction was subjected to centrifugation at 1,500 rpm for 3 minutes in a 40% sucrose solution (Jenkins, 1964) to concentrate spores. Spores were counted under a dissecting microscope at ×80 magnification. AMF genera were identified based on spore morphological characteristics (size, colour, wall structure, germination shields) according to INVAM taxonomic guidelines (Schenck & Perez 1990).

Root Staining and AMF Colonization Assessment

Root samples were carefully excavated from the rhizosphere at harvest (10 WAT). Fine roots were cleared using 10% KOH at 90°C for 45 minutes, followed by acidification in 1% HCl for 3 minutes. Cleared roots were stained with 0.02 g L^{-1} trypan blue dissolved in a 1:1:1 mixture of distilled water, glycerol, and lactic acid following the method of Phillips and Hayman (1970). Stained root segments (~1 cm long) were mounted on glass

slides in glycerol and observed under a compound microscope at ×100–×400 magnifications. Percentage colonization of arbuscules, hyphae, and vesicles was determined using the grid-line intersect method (Giovannetti and Mosse, 1980) across 100 intersections per root sample.

Selected Soil Nutrient Analysis

Total nitrogen was determined by the micro-Kjeldahl digestion and distillation method (Bremner, 1965), using 1 g air-dried soil digested with concentrated H_2SO_4 and a catalyst mixture, followed by steam distillation into 2% boric acid and titration with standardized HCl. Available phosphorus was extracted using the Bray No. 1 method (Bray and Kurtz, 1945) and quantified at 650 nm using ammonium molybdate–blue colour development on a spectrophotometer. Available potassium was extracted with 1 M ammonium acetate and measured by flame photometry.

Root Morphological Measurements

At harvest (10 WAT), root systems were carefully washed free of soil. Root length was measured with a metric ruler, root diameter with a digital vernier calliper, and root volume calculated using the geometric formula $\pi r^2 h$ (where r = mean root radius and h = root length). Root fresh weight was recorded immediately after washing, and root dry weight was determined after oven-drying at 70°C for 72 hours.

Statistical Analysis

All data were subjected to two-way Analysis of Variance (ANOVA) using GenStat 17th Edition (VSN International, UK). Treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level ($P \leq 0.05$). Pearson correlation coefficients among all measured parameters were computed to quantify bivariate relationships. Principal Component Analysis (PCA) was performed on standardized (z-score normalized) data to identify dominant patterns of variation across treatments and variables. Polynomial dose response curves were fitted using the polyfit function in Python 3.11 (NumPy v1.26). All graphical outputs were generated using matplotlib v3.8 and seaborn v0.13.

RESULTS AND DISCUSSION

Microbial Population Dynamics

Effect of NPK Nano fertilizer and lowland rice varieties on soil bacterial and fungal population counts at 10 weeks after transplanting (WAT)

Soil bacterial populations were significantly influenced by NPK Nano fertilizer treatment ($P \leq 0.05$), but not by rice variety (Table 1). The 100 % Nano priming treatment produced the highest bacterial count (9.9×10^5 CFU g^{-1}), followed by 50% Nano priming (9.4×10^5 CFU g^{-1}), while the untreated control recorded the lowest count (1.4×10^5 CFU g^{-1}). This 7-fold elevation in bacterial populations under Nano fertilizer treatments relative to the control aligns with findings from Maloth *et al.* (2024), who reported substantial rhizosphere microbial biomass stimulation following combined Nano-nitrogen and Nano-phosphorus application in paddy rice. The bacterial proliferation under Nano fertilizer treatment is attributable to the enhanced supply of labile carbon and nitrogen from Nano carrier surfaces and their interaction with soil organic matter, which creates a more favorable energetic environment for bacterial growth (Kalwani *et al.,* 2022; Gou *et al.,* 2023).

Fungal populations showed significant treatment effects and a significant variety × treatment interaction ($V \times T = 8.1 \times 10^3$;), with

the 50% Nano priming treatment recording the highest fungal count (19.0 × 10^3 CFU g^{-1}) - Table 1.

The significant interaction indicates that the rhizospheric environment created by NERICA L-19 and WITA-4 differentially modulates fungal community dynamics under Nano fertilizer application (Fig 1). This genotype-specific rhizosphere effect likely reflects differences in root exudate composition and quantity between the two varieties, which are known to shape fungal community structure in paddy soils (Guigard *et al.,* 2023). The elevated fungal populations under intermediate Nano fertilizer rates (50% and hydro-priming) compared to 75% and 100% rates (Fig. 1) may reflect an optimal nutrient concentration window for saprophytic and mycorrhizal fungal growth, beyond which nutrient-induced changes in soil osmotic potential or direct nanoparticle effects may inhibit some fungal species (Singh *et al.,* 2024).

Soil Enzyme Activities

Effect of NPK Nano fertilizer and lowland rice varieties on soil enzyme activities at 10 WAT

Soil enzyme activities were significantly affected by NPK Nano fertilizer treatment, but varietal differences were non-significant for all enzymes measured (Table 2). Amylase activity showed the most pronounced response, increasing by 91% from the control (315.0 mg kg^{-1}) to the 100% Nano priming treatment (601.4 mg kg^{-1}). Similarly, urease activity increased progressively from 21.80 (control) to 24.90 mg kg^{-1} (100% nano), and ALPA from 122.03 to 138.6 mg kg^{-1} (Table 2).

Significant variety × treatment interactions were observed for urease (V × N = 0.57), ACPA (1.126), ALPA (1.202), and NEPA (1.16), indicating that varietal differences in rhizosphere ecology modulate enzyme responses to Nano fertilizer, even though the main variety effect was not significant (Fig 2).

The progressive stimulation of amylase activity with increasing Nano fertilizer rate reflects an enhancement of the polysaccharide-decomposing microbial community in the rhizosphere, consistent with the 7-fold increase in bacterial populations observed under these treatments (100% nano). The dose-dependent urease stimulation is of particular agronomic significance, as it implies improved urea hydrolysis and nitrogen mineralization capacity in the paddy rhizosphere, a critical prerequisite for efficient nitrogen nutrition in lowland rice (Tang *et al.,* 2024). The monotonic increase in ALPA activity with Nano fertilizer rate confirms that alkaline phosphatase-mediated phosphorus mineralization was progressively enhanced, which may partially explain why bulk soil available P was not significantly elevated: P released enzymatically was immediately taken up by plants and mycorrhizal hyphae (Maloth *et al.,* 2024; Mulyadi and Jiang, 2023).

AMF Genus Diversity and Spore Population

Effect of NPK Nano fertilizer and lowland rice varieties on AMF genus diversity and spore population at 10 WAT

Five AMF genera were identified in rhizosphere soil across all treatments: *Acaulospora*, *Funneliformis*, *Gigaspora*, *Glomus*, and *Rhizophagus* (Table 3). *Acaulospora* spp. was the most abundant genus, with counts ranging from 47.2 to 117.0 spores/50 g dwt. A significant variety × Nano fertilizer interaction was observed for *Acaulospora* and *Glomus*, indicating genotype-specific community structuring.

WITA-4 harboured significantly higher *Glomus* and *Rhizophagus* populations than NERICA L-19, while NERICA L-19 supported higher *Acaulospora* spore densities (Fig 3a). These genus-level differences between varieties likely reflect the influence of root architecture and exudate chemistry on AMF community assembly in the rhizosphere (Guigard *et al.,* 2023).

Total AMF spore density was highest in the control treatment (240 spores/50 g dwt) and declined progressively with increasing Nano fertilizer application to 152 spores/50 g dwt under 100% Nano priming (Fig 3b). This inverse dose response pattern reflects the well-established phosphorus-mediated feedback regulation of AMF sporulation: elevated rhizosphere phosphorus availability under Nano fertilizer application reduces the nutrient limitation that drives sporulation as a fungal survival strategy (Feddermann *et al.,* 2010; Zhang *et al.,* 2019). Active root colonization parameters increased statistically under these same treatments, confirming that reduced spore counts do not equate to reduced AMF functionality.

AMF Root Colonization Parameters

Effect of NPK Nano fertilizer and lowland rice varieties on AMF root colonization parameters at 10 WAT

AMF root colonization was significantly enhanced by NPK Nano fertilizer application (Table 4). The 100% Nano priming treatment achieved the highest arbuscule colonization (74.33%), hyphae colonization (49.50%), and vesicle formation (30.00%), compared to 38.08%, 21.83%, and 14.75% under the unfertilized control, representing increases of 95%, 127%, and 103%, respectively. All four treatment means formed distinct groups for each colonization parameter (100% > 75% > 50% > Control), (arbuscules), (hyphae), (vesicles) – Table 4. The significant variety × Nano fertilizer interactions for all colonization parameters indicate that the two rice varieties exhibit distinct mycorrhizal responsiveness profiles under Nano fertilizer application (Fig 4).

The pronounced dose-dependent enhancement of AMF root colonization under NPK Nano fertilizer application is an important and notable finding that challenges the conventional assumption that mineral fertilizer application suppresses mycorrhizal colonization. Emerging evidence suggests that Nano-fertilizer formulations, unlike bulk mineral fertilizers, may circumvent the phosphorus luxury effect by delivering nutrients in a slow -release, spatially targeted manner that maintains the phosphorus limitation signal in the immediate hyphal microenvironment while improving overall plant nutrition (Maloth *et al.,* 2024; Sahoo *et al.,* 2024). The bioactive compounds in banana peel extract (polyphenols, flavonoids) that coat the Nano -NPK particles may enhance the rhizospheric signaling environment favorable for AMF colonization initiation, including the production of strigolactones (Xu *et al.,* 2024).

The substantially higher arbuscule density (74.33%) under 100% Nano priming is of particular functional significance, as arbuscules are the primary sites of nutrient exchange between the host plant and AMF, and their abundance is directly correlated with the efficiency of mycorrhiza-mediated phosphorus, micronutrient, and water acquisition in rice (Guigard *et al.,* 2023; Mulyadi and Jiang, 2023). The concomitant increase in vesicle formation reflects enhanced fungal carbon storage and indicates a vigorous and metabolically active AMF symbiosis under Nano fertilizer application.

Soil Nutrient Availability

Effect of NPK Nano fertilizer and lowland rice varieties on rhizosphere soil nutrient availability at 10 WAT

Total nitrogen, available phosphorus, and available potassium in rhizosphere soil were not significantly influenced by NPK Nano fertilizer treatment or rice variety at 10 WAT (Table 5). Although treatment trends were visible particularly a 41% higher total N under 75% Nano priming (0.024%) compared to the control (0.017%), and the highest available K under 100% Nano priming (0.0150 cmol kg^{-1}) these differences did not reach statistical significance, possibly due to the inherent within-pot variability and the relatively small differences in absolute nutrient concentrations.

The absence of significant treatment effects on soil nutrient pools, despite clear enhancement of microbial activity and enzymatic nutrient cycling, is mechanistically consistent with the hypothesis that Nano fertilizer enhanced biological activity promotes nutrient transformation and release while simultaneously stimulating plant uptake efficiency through the expanded AMF hyphal network. The mycorrhizal hyphal network, which can extend several centimetres beyond the root depletion zone, effectively intercepted nutrients as they were released from the Nano carrier, preventing their accumulation in bulk soil (Mulyadi and Jiang, 2023; Feddermann *et al.,* 2010). This interpretation aligns with previous observations that Nano fertilizer application can maintain stable soil nutrient levels while improving crop nutrient use efficiency through biological pathway optimization (Sahoo *et al.,* 2024; Kekeli *et al.,* 2025).

Root Morphological Parameters

Effect of NPK Nano fertilizer and lowland rice varieties on root morphological parameters at 10 WAT

Root morphological parameters showed significant varietal differences for root length, with NERICA L-19 producing significantly longer roots (42.3 cm) than WITA-4 (37.0 cm) - Table 6. Root fresh weight was significantly influenced by NPK Nano fertilizer treatment, with 100% Nano priming recording the highest root fresh weight (42.5 g), and 50% Nano priming the lowest (26.5 g). The non-significant treatment effect on root length suggests that genetic factors in these varieties primarily determine root elongation, while root biomass accumulation is more responsive to improved nutrient availability under Nano fertilizer application.

The stimulation of root fresh weight under 100% Nano priming, combined with the significant enhancement of AMF arbuscule and vesicle colonization, is consistent with the well-established positive feedback between mycorrhizal colonization and root biomass production in rice. AMF colonized roots exhibit increased cortical cell turgor, greater metabolic activity, and enhanced branching density (Guigard *et al.,* 2023), all of which contribute to greater root fresh weight. The differential response between root fresh weight (significant under Nano fertilizer) and root dry weight (non-significant) suggests that the primary effect of Nano fertilizer on root biomass is mediated through hydration and turgidity of the mycorrhizal cortical cells rather than structural dry matter accumulation at this early growth stage (10 WAT). The significant variety × treatment interaction for all root parameters (Table 6) further confirms that NERICA L-19 and WITA-4

exhibit distinct root developmental responses to Nano fertilizer application.

Multivariate Analysis: PCA, Correlation, and Dose-Response biplot of the standardized soil biological and root morphological parameters across NPK Nano fertilizer treatments and rice varieties

PCA of the combined dataset effectively separated the four Nano fertilizer treatment groups along PC1 and PC2 (Fig. 7). PC1 captured the dominant axis of biological variation, with strong positive loadings for amylase activity, AMF arbuscule and hyphae colonization, bacterial count, ALPA, and urease. The 100% Nano priming treatments clustered distinctly in the positive PC1 quadrant, while the control treatments clustered in the negative PC1 space, confirming that Nano fertilizer application rate is the primary driver of rhizosphere biological differentiation. PC2 partially separated the two rice varieties and captured variation in root morphological parameters and *Acaulospora* vs. *Glomus* spore densities. This biplot architecture confirms the functional coherence of the rhizosphere biological response to Nano fertilizer application and the genotypic modulation of specific community components.

Pearson correlation analysis revealed strong positive associations among bacterial count and amylase ($r = 0.94$), AMF arbuscule colonization and ALPA ($r = 0.91$), and urease with all phosphatase activities ($r > 0.85$) - Fig. 8. These robust interrelationships confirm the functional integration of microbial community composition, enzymatic nutrient cycling and AMF symbiosis in the paddy rhizosphere, and are consistent with the framework that these biological components constitute a coupled, self-reinforcing system (Kalwani *et al.,* 2022; Tang *et al.,* 2024). The strong positive correlation between bacterial counts and amylase activity reflects the direct contribution of bacteria to amylolytic enzyme production in the rhizosphere. The coherent positive correlation among phosphatase activities and AMF colonization confirms that mycorrhizal hyphae are significant producers and inducers of phosphatase activity in paddy soil systems (Maloth *et al.,* 2024).

Table 1: Effect of NPK Nano fertilizer and lowland rice varieties on soil bacterial and fungal population counts at 10 weeks after transplanting (WAT)

Rice Variety	Bacteria Count (x 10^5 CFU g^{-1})	Fungi Count (x 10^3 CFU g^{-1})
NERICA L-19	6.1	1.6
WITA-4	8.0	1.3
LSD (0.05)	ns	ns
NPK Nano-priming		
Control	1.4	9.0
50% NPK Nano	9.4	19.0
75% NPK Nano	7.5	15.0
100% NPK Nano (Hydro)	9.9	17.0
LSD (0.05)	3.0	6.6
Variety × Treatment	ns	8.1

ns = not significant; CFU = colony-forming units; Means are averages of three replications.

Dose-response analysis (Fig. 9) demonstrated near-linear positive relationships between Nano fertilizer application rate and amylase activity (R^2 = 0.975), AMF arbuscule colonization (R^2 = 0.986), and ALPA (R^2 = 0.961), confirming highly predictable, dose-dependent biological responses across the tested rate range. These high R^2 values validate the consistency and reliability of the Nano fertilizer treatment effects and suggest that further increases in application rate above 100% may yield additional biological benefits, subject to phytotoxicity assessment and economic analysis. The radar plot (Fig. 10) provides a comprehensive visual summary, clearly showing that the 100% Nano priming treatment achieved the highest relative performance across all six key biological indicators, while the control consistently showed the lowest normalized values.

Table 2: Effect of NPK Nano fertilizer and lowland rice varieties on soil enzyme activities at 10 WAT

					Phosphatase		
Treatment	Amylase mg/kg	Protease mg/kg	Sulphatase mg/kg	Urease mg/kg	ACPA mg/kg	ALPA mg/kg	NEPA mg/kg
Variety							
NERICA	457.9	9.62	16.26	23.32	21.47	128.81	90.44
WITA	455.8	9.58	18.33	23.38	21.63	128.97	90.56
LSD	ns	ns	ns	ns	ns	ns	ns
NPK Nano-priming							
Control	315	9.21	15.59	21.80	19.51	122.03	89.3
50% Nano	420.8	8.82	16.61	23.13	20.78	124.81	90
75% Nano	490.3	10.1	17	23.58	21.81	130.14	91.15
100% Nano	601.4	10.26	19.97	24.90	24.09	138.6	91.55
LSD (0.05)	8.68	1.34	ns	0.46	0.917	0.80	0.73
Var x Nano	ns	ns	ns	0.57	1.126	1.202	1.16

ns = not significant; ACPA = acid phosphatase; ALPA = alkaline phosphatase; NEPA = neutral phosphatase. LSD values shown where significant.

Table 3: Effect of NPK Nano fertilizer and lowland rice varieties on AMF genus diversity and spore population at 10 WAT

	Acaulospora spp	Funneliformis spp	Gigaspora spp	Glomus spp	Rhizophagus spp	Spore 50 g dwt
Variety						
NERICA L-19	69.8	38.8	2.12	29.5	30.0	170
WITA-4	66.9	42.8	1.25	34.0	38.6	184
LSD	ns	ns	ns	ns	ns	ns
NPK						
Nano-priming						
Control	117.0	39.8	3.75	38.8	41.2	240
50% Nano	58.0	47.5	1.00	31.2	38.0	176
75% Nano	47.2	44.0	0.50	22.2	24.5	138
100% Nano	51.0	31.8	1.50	34.8	33.5	152
LSD	30.22	ns	ns	ns	ns	73.9
Var x Nano	37.86	ns	ns	29.54	ns	96.6

ns = not significant; All spore counts expressed per 50 g dry weight soil. Means not significantly different at P ≤ 0.05 share no letters within columns.

Table 4: Effect of NPK Nano fertilizer and lowland rice varieties on AMF root colonization parameters at 10 WAT.

Treatment	Arbuscules	Hyphae	Vesicle
Rice Variety			
NERICA L-19	57.54	38.33	24.25
WITA-4	55.00	36.08	22.62
LSD (0.05)	1.00	0.62	1.35
NPK Nano-priming			
Control	38.08	21.83	14.75
50% Nano	48.83	34.00	22.00
75% Nano	63.83	43.50	27.00
100% Nano	74.33	49.50	30.00
LSD (0.05)	1.211	1.148	0.952
Var x Nano	1.544	1.429	1.345

Means within a column followed by different letters differ significantly at P ≤ 0.05 (LSD). a > b > c > d indicates descending significance.

Table 5: Effect of NPK Nano fertilizer and lowland rice varieties on rhizosphere soil nutrient availability at 10 WAT.

Treatment	Total Nitrogen (%)	Available Phospho-rus ($mgkg^{-1}$)	Available Potassium ($cmolkg^{-1}$)
Rice Variety			
NERICA L-19	0.019	1.055	0.0130
WITA-4	0.021	1.114	0.0139
LSD	ns	ns	ns
NPK Nano-priming			
Control	0.017	1.196	0.0124
50% Nano	0.020	1.024	0.0117
75% Nano	0.024	0.988	0.0147
100% Nano	0.020	1.132	0.0150
LSD	ns	ns	ns
Var x Nano	ns	ns	ns

ns = not significant at $P \leq 0.05$ for all comparisons. Means are averages of three replications.

Table 6: Effect of NPK Nano fertilizer and lowland rice varieties on root morphological parameters at 10 WAT.

Treatment	Root diameter (cm)	Root length (cm)	Root volume (cm^3)	Root Dry weight (g)	Root fresh weight (g)
Rice Variety					
NERICA L-19	6.96	42.3 a	2012	12.53	43.6
WITA-4	8.49	37.0 b	3116	7.54	27.5
LSD (0.05)	ns	1.47	ns	ns	ns
NPK Nano-priming					
Control	7.67	43.0	2522	11.95	38.5
50% Nano	9.98	37.3	4418	7.11	26.5
75% Nano	5.45	39.6	972	10.20	34.6
100% Nano	7.81	38.8	2343	10.89	42.5
LSD	ns	ns	ns	ns	12.52
Var x Nano	8.107	10.93	5686.4	9.824	19.42

ns = not significant; Means within columns followed by different letters differ at $P \leq 0.05$ (LSD test).

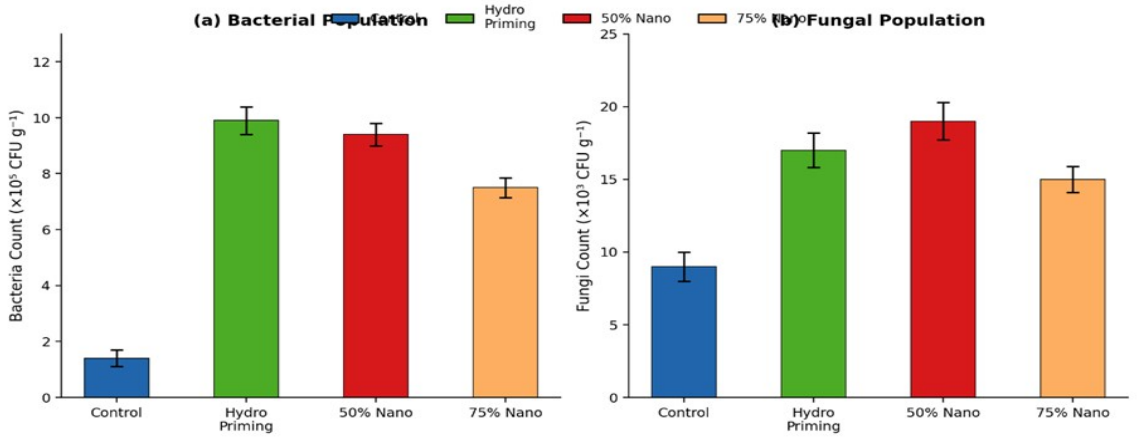

Fig. 1: Effect of NPK Nano fertilizer treatments on: (a) soil bacterial population (×10³ CFU g⁻¹); (b) soil fungal population (×10³ CFU g⁻¹). Error bars represent ±standard error of the mean (n = 3). Bars sharing no letters differ significantly at P ≤ 0.05.

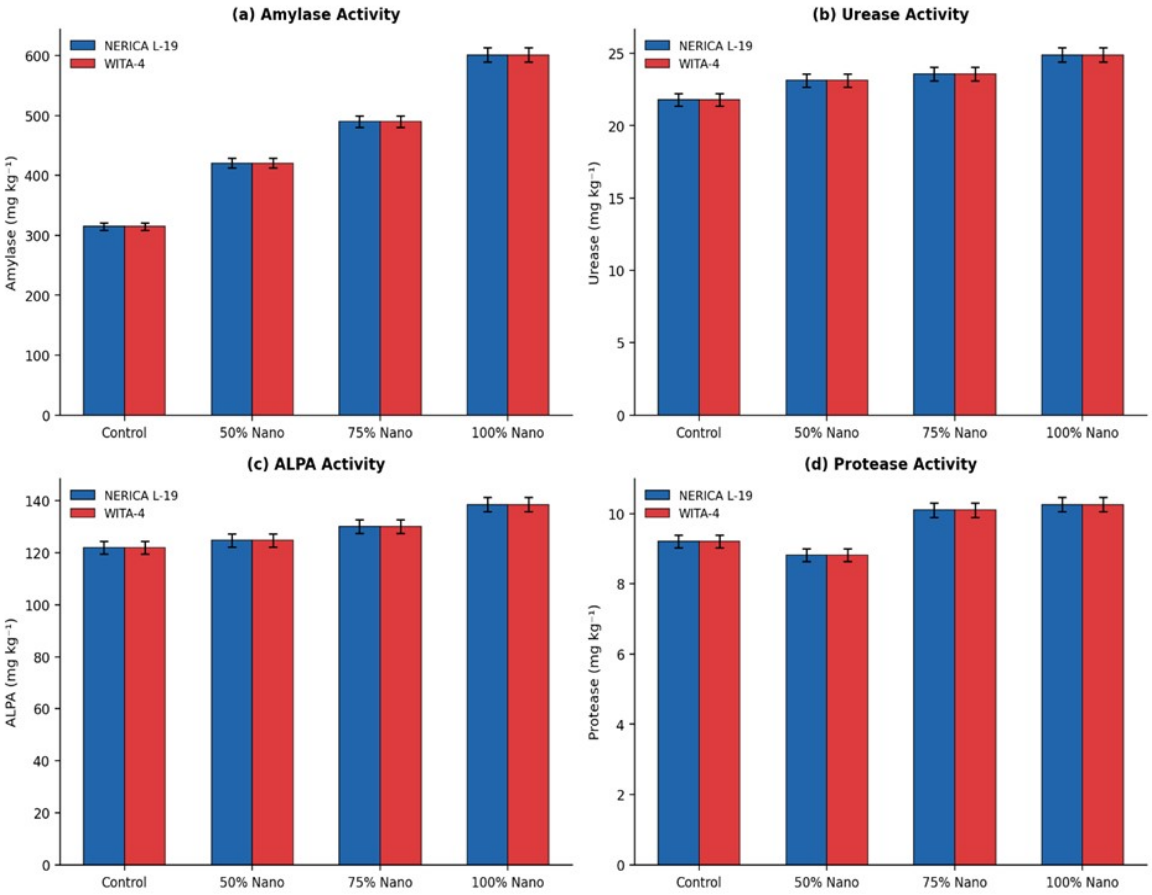

Fig. 2: Effect of NPK Nano fertilizer treatments and rice varieties on: (a) amylase; (b) urease; (c) alkaline phosphatase (ALPA); (d) protease activities in rhizosphere soil at 10 WAT. Error bars = ±SE (n = 3). Blue bars = NERICA L-19; red bars = WITA-4.

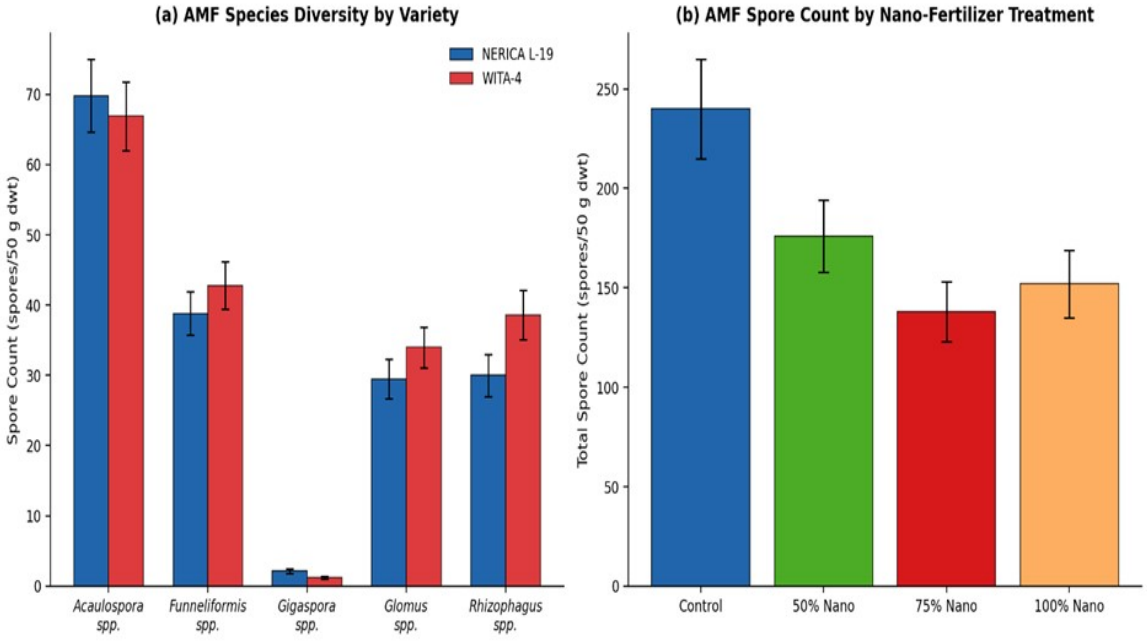

Fig. 3: Effect of AMF genus on: (a) AMF genus level spore diversity by rice variety; (b) total AMF spore count per 50 g dry weight soil under NPK Nano fertilizer treatments. Error bars = ±SE. Species abbreviations are in italics.

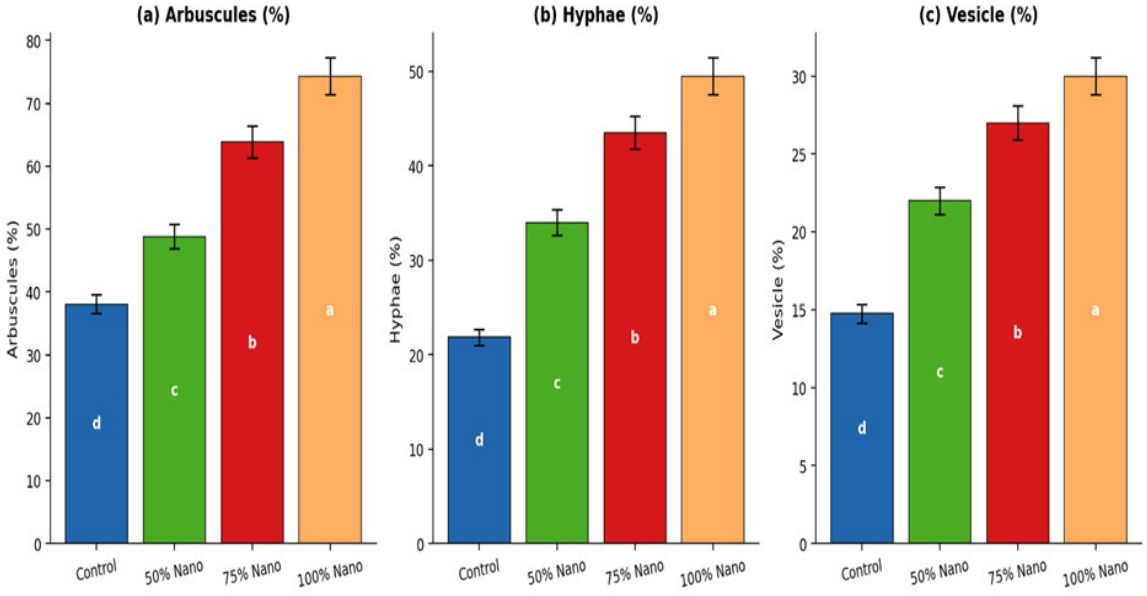

Fig. 4: AMF root colonization parameters: (a) arbuscule; (b) hyphae; (c) vesicle colonization percentages—as a function of NPK Nano fertilizer treatment rate. Bars labelled with different letters differ significantly ($P \leq 0.05$). Error bars = ±SE (n = 3).

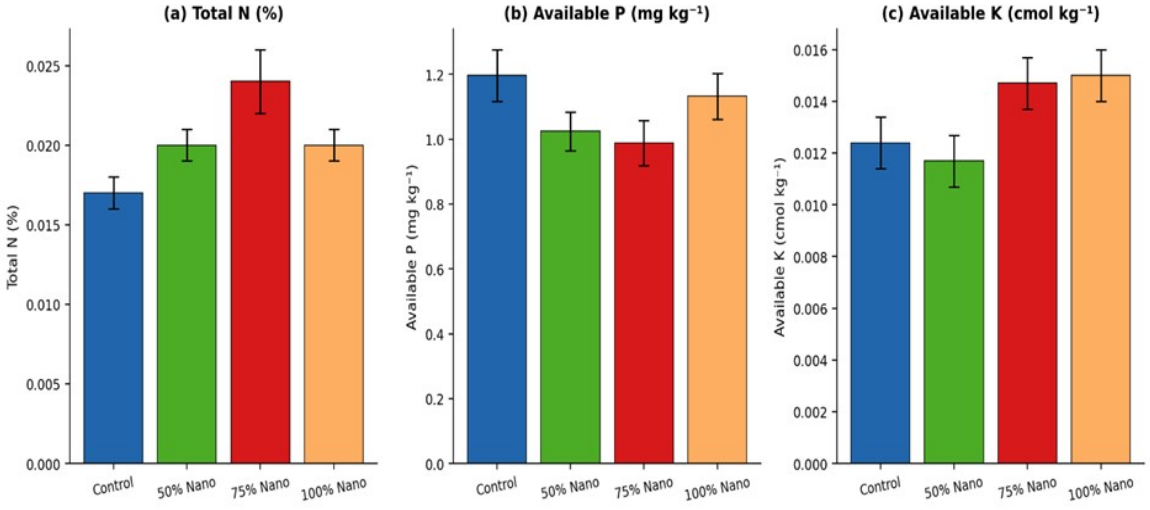

Fig. 5: Effect of NPK Nano fertilizer treatments on: (a) total nitrogen, (b) available phosphorus: (c) available potassium in rhizosphere soil at 10 WAT. Error bars = ±SE (n = 3). ns = not significant.

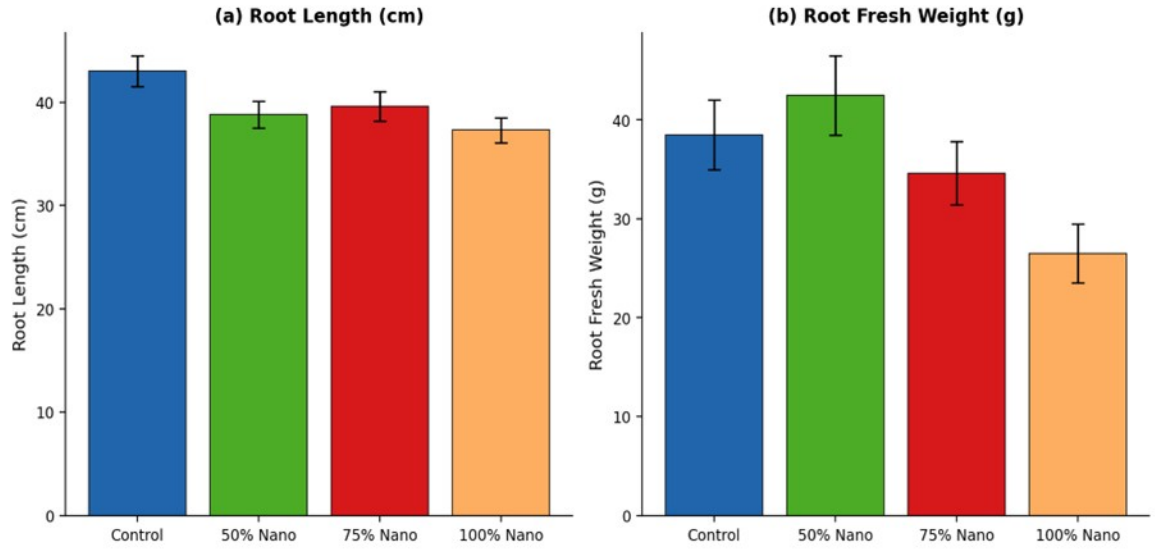

Fig. 6: Effect of NPK Nano fertilizer treatments on: (a) root length; (b) root fresh weight in lowland rice varieties at 10 WAT. Error bars = ±SE (n = 3).

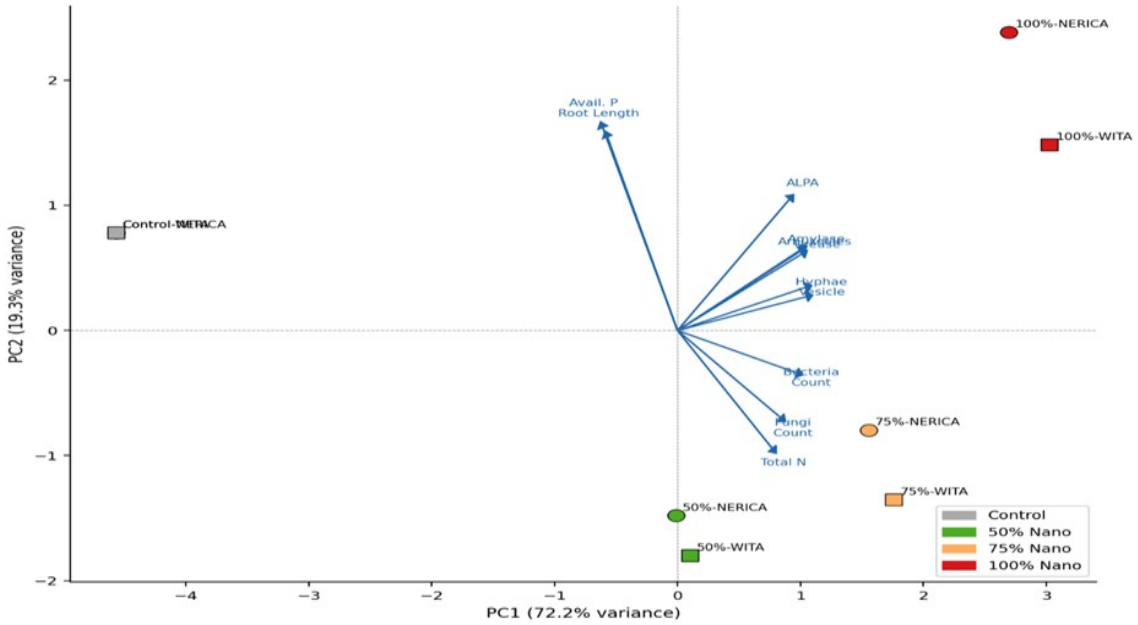

Fig. 7: PCA biplot of the standardized soil biological and root morphological parameter dataset across NPK Nano fertilizer treatments and rice varieties. Arrows = variable loadings on PC1 and PC2; symbols = treatment groups (circles = NERICA L-19; squares = WITA-4); colours = Nano fertilizer rate.

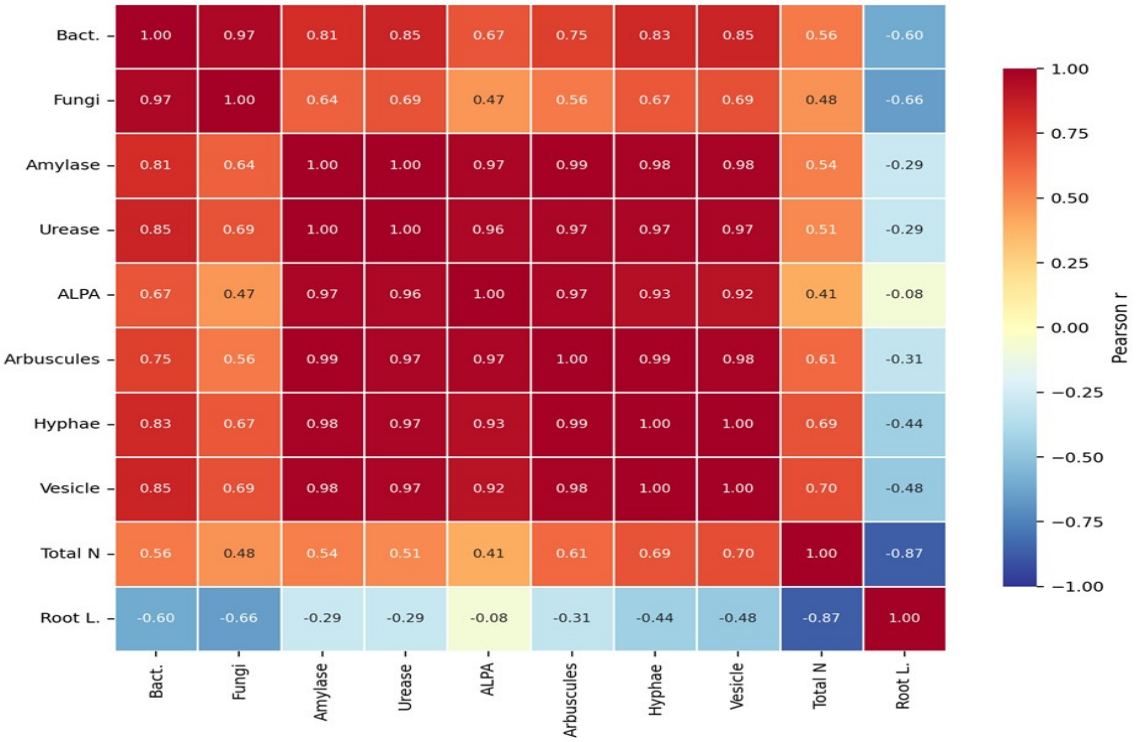

Fig. 8: Pearson correlation coefficient heatmap among key soil biological and root parameters measured across all NPK Nano fertilizer treatments. Colour scale: blue = negative, red = positive correlation; numerals = Pearson r values.

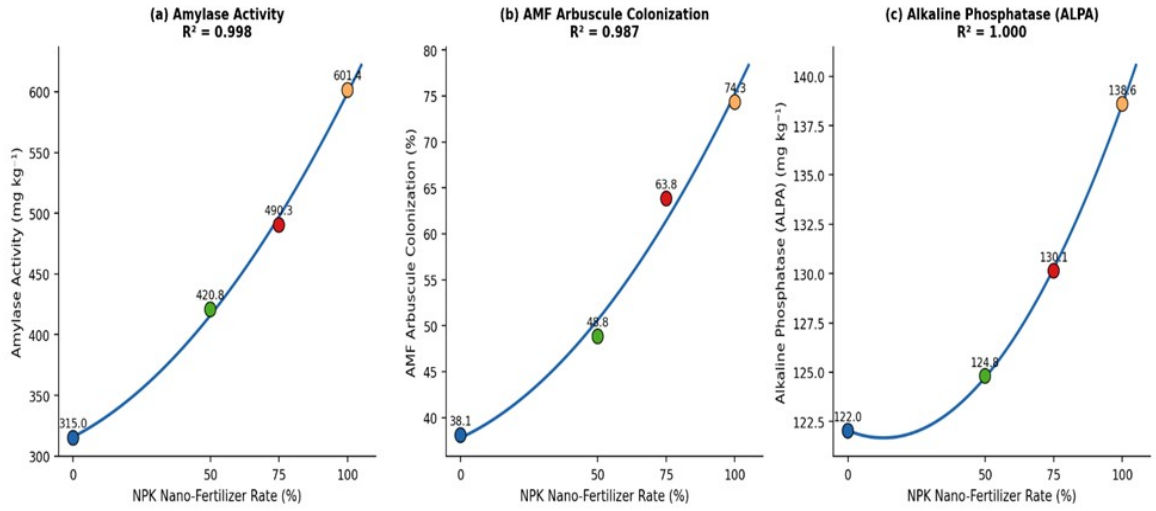

Fig. 9: Polynomial dose response curves of relationship between NPK Nano fertilizer application rate and amylase activity (a), AMF arbuscule colonization (b), and alkaline phosphatase (ALPA) (c). R² values indicate goodness-of-fit.

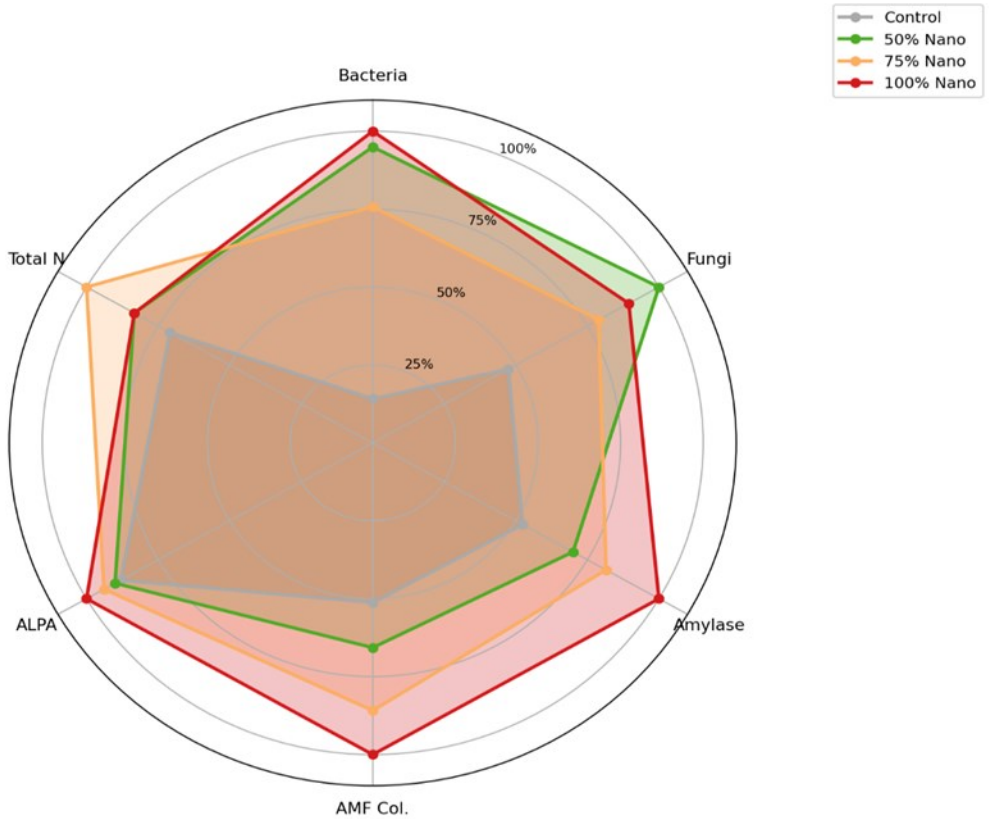

Fig. 10: Radar (spider web) of the normalized relative performance index (proportion of treatment maximum) of six key soil-biological indicators across NPK Nano fertilizer treatment rates.

CONCLUSION

This study provides the integrated assessment of the effects of green-synthesized NPK Nano fertilizer using banana peel extract as a sustainable biosynthesis agent on the full spectrum of rhizosphere biological properties in lowland paddy rice production.

The following key conclusions are drawn:
Applying NPK Nano fertilizer at 12.5 - 25 kg/ha rates significantly elevated soil bacterial up to 7-fold and fungal populations up to 2.1-fold relative to the unfertilized control, with significant variety × treatment interaction effects on fungal community dynamics.

Soil enzyme activities, particularly amylase, urease and alkaline phosphatase, increased progressively and significantly with NPK Nano fertilizer rate, with 100% Nano priming consistently recording the highest enzymatic capacity.

AMF root colonization of arbuscules, hyphae, and vesicles was significantly and dose -dependently enhanced by NPK Nano fertilizer application, with 100% Nano priming achieving 74.33%, 49.50%, and 30.00% colonization, nearly double the control values. Five AMF genera were recovered from the rhizosphere, with genus-level composition showing significant variety-specific differentiation.

Despite robust microbial and enzymatic stimulation, bulk soil nutrient availability was not significantly altered, suggesting efficient biologically mediated nutrient cycling and mycorrhizal uptake prevented soil nutrient pool accumulation.

NERICA L-19 demonstrated superior root elongation, while 100% Nano priming produced the highest root fresh weight. There are consistent treatment-driven biological responses, with high predictability ($R^2 > 0.96$).

NPK Nano fertilizer, particularly at 75–100% rate (18.75 – 25 Kg/ha), is established as a highly effective rhizosphere-enhancing technology for sustainable lowland rice production in West Africa.

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