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RESEARCH ARTICLE

Partial substitution of inorganic fertilizer with nano fertilizer improved soil microbial diversity and yield of irrigated lowland rice

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Abstract

Nanotechnology holds an immense potential to revolutionize rice production especially in enhancing grain yield. This study evaluates the effect of nanotechnology on grain yield, microbial activity, root development and economic returns over multiple cropping seasons (2022 to 2024). In the 2022 Wet Season (WS), while full-dosed inorganic fertilizer (100 % Rice Crop Manager, RCM) (6.87 tha^{-1}) did not meet the targeted yield due to typhoon-induced lodging, treatments with 50 % RCM combined with Nano fertilizer (NF) produced comparable yields (6.27 to 6.62 tha^{-1}). In 2023 Dry Season (DS), both full RCM and 50 % RCM with NF yielded 6.85 t ha^{-1}; in 2023 WS, 50 % RCM with NF yielded higher yield (4.88 to 5.60 tha^{-1}) than no-fertilizer control (4.35 tha^{-1}); and in 2024 DS, full RCM produced the highest yield of 8.51 tha^{-1} while 50 % RCM + NF yielded 7.11 tha^{-1}. Soil microbial activity, using Shannon Index was lower in plots with continuous synthetic fertilizer application (H'=2.71) in 2024 DS, showing a decline in soil microbial diversity. Root system development was most robust with full RCM, but the reduced RCM with NF also had favourable root growth, particularly lateral root length. Partial budget analysis revealed that while full RCM provided the highest gross income, the 50 % RCM + NF produced yielded higher net incomes due to lower input costs. The promising results of reduced RCM rates + NF warrant further investigation to assess long-term sustainability, cost-effectiveness and broader environmental impact.

Keywords: grain yield; nanotechnology; rice; root development; soil microbial diversity

Introduction

Environmental concerns and rising chemical fertilizer costs highlight the need for alternative technologies such as nano fertilizers, biofertilizers and bio-inoculants to boost crop yields. Nanotechnology is currently widely used in modern agriculture and is recognized as the new frontier in precision agriculture (1). Nano fertilizers are being investigated as a method of increasing nutrient efficiency and improving plant nutrition when compared to regular fertilizers (2). Nanoparticles, with their high sorption capacity, higher surface to volume ratio and controlled -release kinetics to specified areas, have the potential to enhance plant growth. Because of this, nano-structured fertilizers can be employed as a smart nutrient delivery system for plants. Nano fertilizers are released at a much slower rate compared to traditional fertilizers. This method enhances nutrient management, specifically by boosting nutrient-use efficiency (NUE) and reducing the leaching of nutrients into groundwater (3). As a result, the increased NUE and significantly lower nutrient losses associated with nano fertilizers contribute to enhanced productivity (6-17 %) and improved nutritional quality of agricultural crops (4).

Additionally, nano fertilizers rejuvenate soil health by fostering the activity of both micro and microorganisms within the soil. This leads to the production of enzymes and compounds, the formation of organic matter, the transformation of chemicals into accessible nutrients for plants, the balancing of pH levels and the removal of heavy metals, which in turn supports the habitat for soil organisms especially earthworms. Furthermore, it has been shown to significantly speed up the composting process of waste materials.

Nano fertilizer comprises components that help maintain equilibrium in the soil. It supplies essential elements for the naturally existing soil microorganisms to flourish and proliferate rapidly. This formulation is an organic mix of micronutrients, enzymes and trace minerals. It includes plant-derived vitamin precursors in a highly concentrated form of autotrophic, aerobic and facultative enzymes, coenzymes and exoenzymes. This system is designed to stimulate microorganisms, featuring inducer molecules that enhance the activity and replication rate of bacteria, fungi, algae, actinomycetes and others. It also contains glycosides, which deliver the necessary energy to support heightened microbial activity. A greenhouse study revealed that utilizing a combination of traditional inorganic fertilizers and nano fertilizers significantly improved various agronomic factors, including plant height, number of reproductive tillers, panicle count, spikelet quantity and overall grain weight, with increases of 3.6 %, 9.10 %, 9.10 %, 15.42 % and 17.5 %, respectively (5). In addition, nano-chelating technology can reduce the reliance on chemical fertilizers and has the potential to enhance crops with essential micronutrients (6).

The possibility of minimizing the use of inorganic fertilizers paired with nano fertilizers to maintain optimal yields in irrigated lowland rice is highly significant. The overall aim of this research is to assess the effectiveness of nano fertilizers within a high yielding irrigated lowland rice context. The specific aims are [1] to examine the impact of utilizing nano fertilizers in conjunction with prescribed inorganic fertilizers on the growth and yield of irrigated lowland rice, [2] to evaluate the influence of nano fertilizers on soil fertility, microbial diversity and root system development and [3] to analyze the economic advantages of employing nano fertilizers.

Materials and Methods

The experimental field and setup

The field trial was established in Wet Season 2022 to Dry Season 2024 at the Philippine Rice Research Institute, Central Experiment Station (PhilRice CES), Maligaya, Science City of Muñoz, Nueva Ecija (15.67171°N 120.89371°E). The soil was classified as Maligaya Series having a very fine texture (clay) that shrink and swell during drying and wetting cycles. This area is highly suitable for rice production with Type I climate characterized with two distinct dry (December to May) and wet (June to November) seasons.

The test variety was NSIC Rc222, a high-yielding inbred rice, with an average yield of 6.1 t/ha and a maximum yield of 10 t/ha that matures in 114 days. The experiment was laid out in a Randomized Complete Block Design with four blocks and six treatments (Fig. 1). The data was analyzed using the Statistical Tool for Agricultural Research and treatment means were compared using the Tukey's Honest Significant Difference test at 5 % probability level.

Fertilizer treatments

The recommended inorganic fertilizer rate was based on the recommendation output of the Rice Crop Manager (RCM) App with a target yield specific to the experimental site. The target yield setting was based on the extracted information on the location of the field, type of variety to be planted (type and duration), typical yield from the past seasons in the site, sowing month, type of seeds and the status of water supply during the cropping season.

The recommended fertilizer rate generated from the RCM App was 143-28-28 kg N, P_2O_5 and K_2O per hectare with a corresponding dry season target yield of 6.85t/ha. The generated fertilizer recommendation was assigned as Treatment 1 or the 100 % RCM. Treatments 2 to 5 were the Nano fertilizer (NF) treatments combined with 50 % RCM reduced rate while Treatment 6 was assigned as no fertilizer treatment. Treatments with NF differed on the amount and time of application. Treatment 2 has 15 g/ha NF equally applied during the seed soaking, at seedling stage and at 30 days after transplanting (DAT). Treatment 3 has 15 g/ha NF applied at seed soaking (5 g) and as soil treatment (10 g) applied a day before transplanting. Likewise, Treatment 4 is like Treatment 3. However, the 5 g NF is applied at 30 DAT. Moreover, Treatment 5 has 20 g/ha NF applied at seed soaking (5 g), as soil treatment (10 g) and at 30 DAT (5 g). For seed treatment, 5 g of NF powder was activated in 1 L non-chlorinated water for 48 hr and was diluted to 15 L of non-chlorinated water prior to seed soaking. The seeds were soaked in the solution for 24 hr at a rate of 15 L solution per 20 kg seeds. After seed soaking, the seeds were incubated for 24 hr prior to seed sowing. The same procedure was followed for the activation and dilution for both foliar (30 DAT) and soil treatments, though the volume of non-chlorinated water were proportioned to the amount of NF powder used. The fertilizer treatments are shown in Table 1.

Crop management

All other crop management except for fertilizer application were the same among treatments. Twenty-one-day old rice seedlings were manually transplanted at 20 cm by 20 cm between hills and rows. The area was sprayed with pre-emergence herbicide at saturated soil condition three days after the crop was established and was followed by spot weeding thereafter. Irrigation water was maintained at appropriate levels during critical stages and was drained two weeks before harvest.

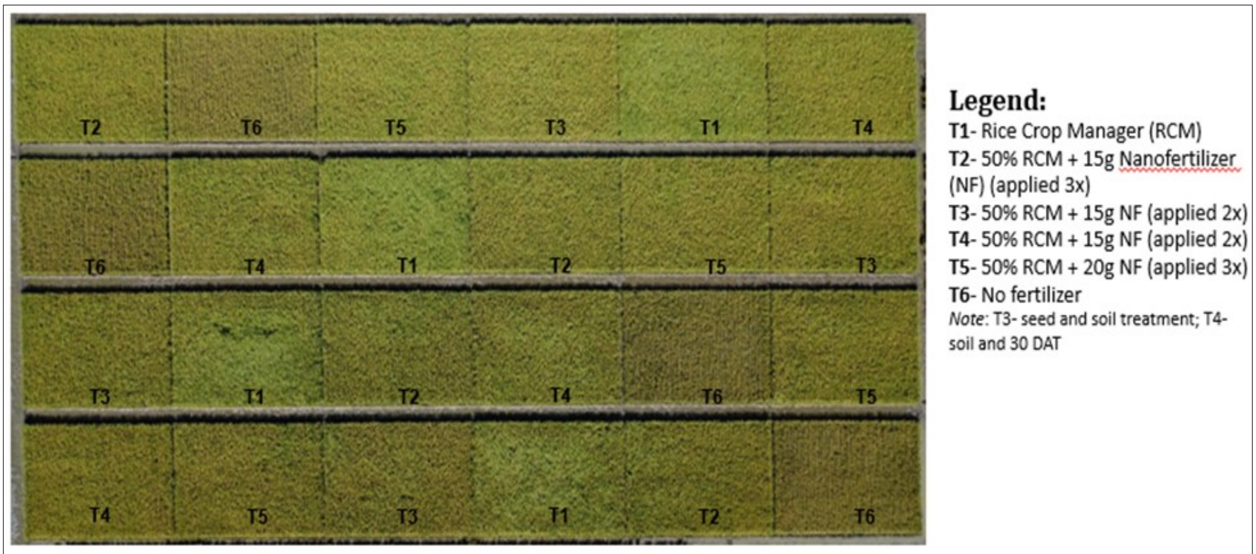

Fig. 1. Field experimental area at PhiliRice Central Experiment Station.

Table 1. Fertilizer treatments and the recommended fertilizer rate used in the field trial. 2022 Wet Season to 2024 Dry Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

Fertilizer Treatments (T)	**Fertilizer Rate** ($N-P_2O_5-K_2O$ kg/ha)
T1. Rice Crop Manager (RCM)	143-28-28
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	71.5-14-14 + 15g/ha NF
T3. 50 % RCM + 15g NF (applied 2x)	71.5-14-14 + 15g/ha NF
T4. 50 % RCM + 15g NF (applied 2x)	71.5-14-14 + 15g/ha NF
T5. 50 % RCM + 20g NF (applied 3x)	71.5-14-14 + 20g/ha NF
T6. No fertilizer	0-0-0

Note: T3- seed and soil treatment; T4- soil and 30 DAT

Determination of soil microbial functional diversity

This analysis will provide information on the microbial functional diversity in the area as affected by the treatments. Soils were collected from PhilRice Central Experimental Station, Block 6 Lot 4. The area has six treatments per block with 4 replications laid out in a Randomized Complete Block Design (RCBD).

1 g of peptone powder was poured into the 1L of water and mixed well until the powder was dissolved. Peptone-water solution was transferred into each Gerber test bottle (90 mL and 99 mL). Three subsamples were collected per plot following a zigzag pattern. About a kg of soil sample was collected from the top 0-6 cm. Each sample was placed in a clean Ziplock bags. The collected samples were processed immediately for isolation of bacteria. 10 g of soil sample were weighed and were used for the isolation. The weighed-soil was transferred to a 90 mL peptone water and manually shaken. One mL aliquot of the appropriate dilution was transferred to 99 mL peptone water using a pipettor. The 150 µL soil-peptone-water was dispensed into BiOLOG Ecoplate using the multichannel pipettor and read using the Microplates Microstation reader. Plates were incubated at 25 °C after every reading. Readings were done from Day 0 to Day 7 (Fig. 2-3).

Evaluation and characterization of root system development

This analysis determines the rice root system development as affected by the treatments. Evaluation and characterization of root system development can be associated to the efficiency of nutrient absorption which can be translated to biomass production and yield. Seventy-two root samples were taken from the experimental area for the root analysis.

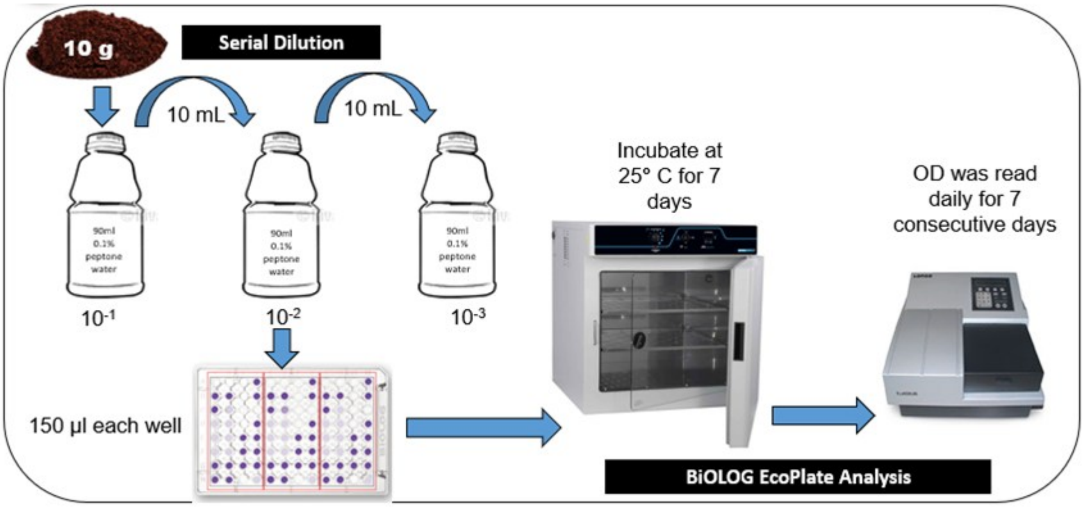

Fig. 2. Assessment of soil microbial functional diversity using BiOLOG EcoPlates.

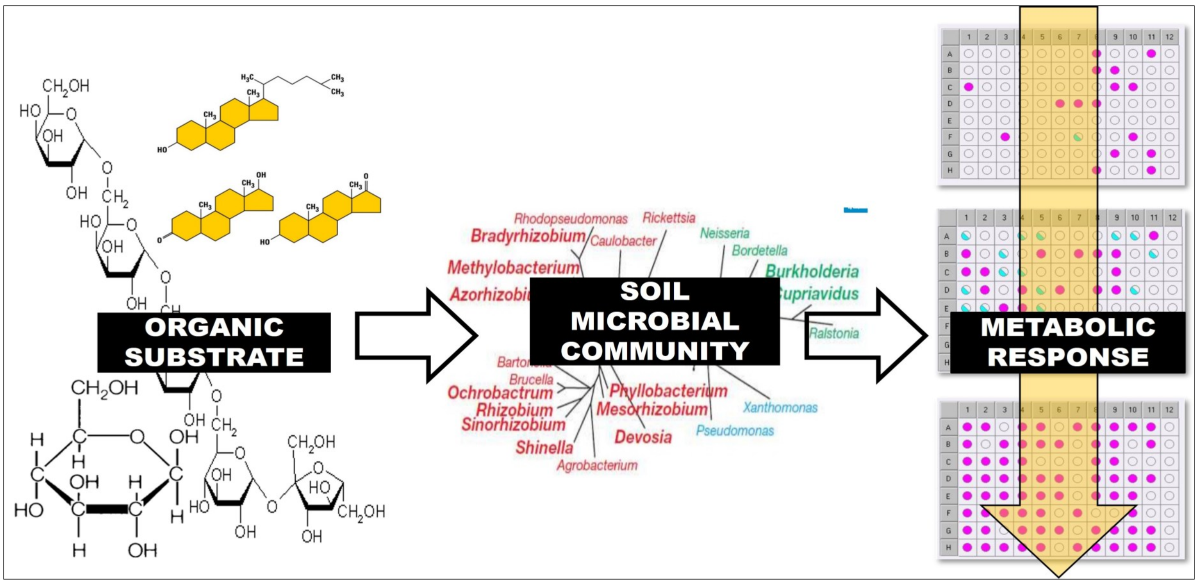

Fig. 3. Community-level physiological profiling to assess soil microbial functional diversity.

At maturity, three plants per entry were sampled for roots with a round monolith method by using a stainless cylinder of 15 cm in diameter and the soil cores of 20 cm in depth were taken. The sampled roots were carefully washed to remove the soil and other dirt particles. The extracted roots were stored in 95 % ETOH for the measurements of total root length, total nodal root length and number of nodal roots. Thereafter, the roots were scanned at 600 dpi using EPSON v700 perfection (dual lens scanner). Scanned images were analyzed for total root length (TRL) using WinRhizo v. 2007d (Regent Instruments, Quebec, Canada). A pixel threshold value of 200 was set for the root length analysis. The total nodal root length (TNRL) was computed as the difference between TRL and TLRL. After scanning, the root samples were oven dried at 70 °C for 48 hr before the root dry weight (RDW) was recorded.

Partial budget analysis

A simple partial budget analysis was conducted to assess the economic benefits of fertilizer treatments. This was the difference between the total cost of fertilizers used for each treatment and the gross income gained for each treatment. The gross income was calculated as the total monetary value of the grain yield multiplied by the average price of dry palay using Philippine Statistics Authority as reference. The price per bag of Complete or 14-14-14 and Urea or 46-0-0 fertilizers were sourced out from the Department of Agriculture - Fertilizer and Pesticides Authority average prices of 6 major grades of fertilizer in Region III. All other production costs were assumed equal among the treatments.

Results and Discussion

Grain yield

In 2022 Wet Season, the targeted grain yield (7.0t/ha) based on the RCM recommendation for the cropping season was not achieved due to lodging caused by typhoon particularly in the full-dosed inorganic fertilizer treatment. Data on grain yield showed no significant differences between the RCM and NF treatments with 50 % reduced RCM rate, though yield achieved in untreated plots were statistically similar to the use of 50 % reduced inorganic fertilizer rate combined with 15 g and 20 g NF applied twice and thrice, respectively (Table 2). Higher yields obtained from the untreated plots were associated to the high indigenous nutrient supplying capacity of the soil.

It was also noted that the NF combined with 50 % reduced inorganic fertilizer did not lodge during the event of "Typhoon Karding" on September 25, 2022 and was harvested 9 days after. This had contributed to higher percentage of filled spikelets relative to the RCM treatment. On the other hand, 80 % of the crops lodged at a crop angle lower than 45 ° in the RCM plots and were harvested 12 days after the typhoon. Moreover, treatments with NF produced numerically heavier grains and more panicles than the RCM treatment except for 50 % RCM + 15g NF applied during seed soaking and as soil treatment (T3) which had a slightly lower panicles produced. It was also observed that the combination treatments mature a week earlier than the RCM treatment. In general, the combination treatments had a better crop stand after the typhoon. This showed that optimizing crop nutrient requirement through the application of pure inorganic fertilizer in the wet season may not always result in the attainment of optimum yield as abiotic factors such as flood and typhoon can cause potential damage to the grains, thus significantly reducing the yield. Hence, the use of NF combined with 50 % reduced rate of inorganic fertilizer is a viable option that translates into a reasonable yield.

In wet season of the following year (2023), the targeted grain yield (7.0t/ha) based on the RCM recommendation for the cropping season was also not achieved. Data on grain yield showed no significant differences between the No Fertilizer (Control) and NF treatments with 50 % reduced RCM rate. Moreover, yield achieved in plots treated with 100 % RCM is not statistically different than yield achieved in treatments with NF with 50 % reduced RCM with 15 g applied twice and 20 g NF applied thrice. Treatment 1 to Treatment 5 demonstrate a higher yield when compared to plots that no fertilizer applied (T6) (Table 2). In terms of computation the percent increase, it showed that full RCM (T1) has 18.69 % in grain yield than plots with no fertilizer applied (T6) and plots applied with NF with 50 % RCM applied twice or thrice (T2-T5) has of 18.70 %.

In 2023 Dry Season, the targeted grain yield (6.85t/ha) based on the RCM recommendation for the cropping season was achieved particularly in the full-dosed inorganic fertilizer treatment. However, targeted yield was also achieved in the crops applied with the combination of 50 % RCM and NF. Data on grain yield showed a significant difference between the RCM and NF treatments with 50 % reduced RCM rate. Yield in untreated plots recorded a lower yield among the treatments with an average of 3.82 tons/ha. Furthermore, the combination treatments (50 % RCM and NF) had a better crop stand compared to the RCM alone. Heavier grains and a greater

Table 2. Effect of fertilizer management on the yield (t ha^{-1}) of rice. PhilRice Central Experiment Station, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines. 2022 Wet Season to 2024 Dry Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

TREATMENTS	2022 Wet Season	2023 Dry Season	2023 Wet Season	2024 Dry Season
T1. RCM	6.87a	8.37a	5.35	8.51^{a}
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	6.62a	7.50b	5.60	6.89^{b}
T3. 50 % RCM + 15g NF (applied 2x)	6.44ab	7.76b	5.35	6.88^{b}
T4. 50 % RCM + 15g NF (applied 2x)	6.36ab	7.85b	5.55	7.11^{b}
T5. 50 % RCM + 20g NF (applied 3x)	6.27ab	7.51b	4.88	6.82^{b}
T6. No fertilizer	5.91b	3.82c	4.35	4.08^{c}
CV (%)	*4.27*	9.69 %	*21.35 %*	*5.66 %*

Note: Means with the same letter are not significantly different from each other at 5 % level of probability according to the Tukey's Honest Significant Difference (HSD) Test. ns = not significant. T3- seed and soil treatment; T4- soil and 30 DAT number of panicles were numerically produced in the crops fertilized with full inorganic fertilizer followed by treatments with half-dose of inorganic fertilizer plus NF applications.

On the following dry season in 2024, the targeted yield based on RCM Recommendation for the cropping season (7 tha^{-1}) were achieved by plots with full fertilizer applied (T1) (8.51 tha^{-1}) and plots treated with 15 g of NF plus 50 % RCM applied thrice (T4) (7.11 tha^{-1}). However, the lowest grain yield was observed in plots with no fertilizer applied (T6). The data on grain yield applied with 15 g of NF with combination of 50 % RCM ranges from 6.82 to 6.89 tha^{-1}(Table 2). Nano fertilizer has a potential in increasing grain yield (7, 8).

Related studies of on nano fertilizer showed that treatments with 100 ppm and 150 ppm nano calcite at recommended rate and 25 % over the recommended soil fertilizer levels produced varying maximum yields and panicle lengths (9) . These findings suggest that rice yield can be increased with the application of 100 ppm nano calcite foliar fertilizer with recommended level of soil fertilizer. Calcite foliar fertilizer can be instantly absorbed by the plant and broken down into CaO and CO_2, per the product specifications.

Community level physiological profile

Microbial activity in each microplate was expressed as Average Well Color Development (AWCD). AWCD is relevant to sole-carbon source utilization patterns of microbial communities determined by the overall color development in the EcoPlate. It represents the overall metabolic activity of microbial communities, furthermore it estimates the degree of microbial functional diversity based on physiological parameters (10). Carbon substrate utilization showed that addition of the recommended amount of fertilizer and augmenting the nitrogen requirement of the crop based on morphological symptoms modified the metabolic profile of microbial communities. Results indicate complete color development after 120 hr incubation period (11).

Table 3 shows the analysis of variance of AWCD at the 168 hr incubation period revealing significant differences among the six different fertilizer management in 2023 Dry Season. The highest AWCD from 24 hr to 168 hr incubation period was observed in plots fertilized with 50 % RCM and NF. Moreover, curve integration approach using the area under the kinetic curve and Riemann's sum was conducted to take into account the variability in carbon utilization through time. Plots treated with 50 % recommended fertilizer rate based on RCM recommendation in combination with the NF applied one-day before transplanting as soil treatment and at 30 DAT had the highest Reimann's Sum (93.09477) and Area under Kinetic curve (372.3791) relative to other treatments. This indicates higher microbial activity. On the other hand, lowest observations were observed in the control treatments: Reimann's Sum (73.11374) and Area under Kinetic curve (292.455). This indicates lower microbial activity or carbon utilization. During the Dry Season of the following year (2024), the highest value in AWCD from 24 hr to 168 hr incubation was observed in plots treated with 15 g of NF plus 50 % RCM (T2) at Day 1 and at Day 7 respectively, which was also associated with the highest Reimann's Sum (74.92) and Area under the Kinetic Curve (299.69). In addition, the presence of earthworms was also observed. Earthworms influence the soil for sustainable production for soil land management and increase soil fertility and profitable (12). Data on AWCD of Treatments 3 to 5 ranges from 0.36 to 0.50 on Day 1 and 1.32 to 2.07 at Day 7. However, the lowest AWCD, Reimann's Sum and Area under the Kinetic Curve were recorded in plots treated with 15 g of NF (T5) (Table 4).

In 2023 Wet Season, the highest AWCD from 24 hr to 168-hour incubation period was observed in plots fertilized with 50 % RCM and NF (Table 5). Moreover, curve integration approach using the area under the kinetic curve and Riemann's sum was conducted to consider the variability in carbon utilization through time. Plots treated with 50 % recommended fertilizer rate based on RCM recommendation in combination with 15g of NF applied in seed, seedbed and before transplanting as soil treatment and at 30 DAT had the highest Reimann's Sum (74.92284) and Area under Kinetic curve (299.6914) relative to other treatments. On the other hand, lowest observations were observed in treatment 5 with 50 % recommended fertilizer rate combination with 20 g of NF applied in seed, soil as soil

Table 3. Average Well Color Development and Curve integrated variables (Reimann's Sum and Area Under Kinetic Curve) of different fertilizer management representing relative degree of sole carbon source utilization 2023 Dry Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

Treatment	AWCD				Reimann's Sum	Area under the Kinetic Curve
	Day 1	Day 3	Day 5	Day 7		
T1. RCM	1.30^{bc}	2.49^{a}	2.37^{a}	1.63^{b}	83.64194	334.5677
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	1.38^{b}	2.42^{a}	2.41^{a}	1.57^{b}	83.95716	335.8286
T3. 50 % RCM + 15g NF (applied 2x)	1.41^{b}	2.45^{a}	2.53^{a}	2.65^{a}	92.16213	368.6485
T4. 50 % RCM + 15g NF (applied 2x)	1.62^{a}	2.45^{a}	2.53^{a}	2.31^{a}	93.09477	372.3791
T5. 50 % RCM + 20g NF (applied 3x)	1.02^{d}	2.03^{b}	2.15^{b}	2.45^{a}	77.13406	308.5363
T6. No fertilizer	1.17^{cd}	2.01^{b}	2.13^{b}	1.57^{b}	73.11374	292.455

Note: T3- seed and soil treatment; T4- soil and 30 DAT

Table 4. Average well color development and curve integrated variables (Reimann's Sum and Area Under Kinetic Curve) of different fertilizer management representing relative degree of sole carbon source utilization 2024 Dry Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

Treatment	AWCD				Reimann's Sum	Area under the Kinetic Curve
	Day 1	Day 3	Day 5	Day 7		
T1. RCM	0.64^{ab}	1.55^{abc}	1.94^{a}	2.30^{a}	63.2967	253.1868
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	1.05^{a}	1.92^{a}	2.10^{a}	2.34^{a}	74.9228	299.6914
T3. 50 % RCM + 15g NF (applied 2x)	0.50^{b}	1.42^{bc}	1.62^{ab}	2.07^{ab}	54.9306	219.7226
T4. 50 % RCM + 15g NF (applied 2x)	0.46^{b}	1.19^{cd}	1.36^{bc}	1.69^{bc}	46.3686	185.4743
T5. 50 % RCM + 20g NF (applied 3x)	0.36^{b}	0.82^{d}	1.02^{c}	1.32^{ab}	34.3055	137.2219
T6. No fertilizer	1.02^{a}	1.88^{ab}	1.95^{a}	2.08^{ab}	70.7025	282.8101

Note: T3- seed and soil treatment; T4- soil and 30 DAT

Table 5. Average well color development and curve integrated variables (Reimann's Sum and Area Under Kinetic Curve) of different fertilizer management representing relative degree of sole carbon source utilization. 2023 Wet Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

Treatment	AWCD				Reimann's Sum	Area under the Kinetic Curve
	Day 1	Day 3	Day 4	Day 7		
T1. RCM	0.64^{ab}	1.55^{abc}	1.94^{a}	2.30^{a}	63.29671	253.1868
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	1.05^{a}	1.92^{a}	2.10^{a}	2.34^{a}	74.92284	299.6914
T3. 50 % RCM + 15g NF (applied 2x)	0.50^{b}	1.42^{bc}	1.62^{ab}	2.07^{ab}	54.93065	219.7226
T4. 50 % RCM + 15g NF (applied 2x)	0.46^{b}	1.19^{cd}	1.36^{bc}	1.69^{bc}	46.36858	185.4743
T5. 50 % RCM + 20g NF (applied 3x)	0.36^{b}	0.82^{d}	1.02^{c}	1.32^{c}	34.30548	137.2219
T6. No fertilizer	1.02^{a}	1.88^{ab}	1.95^{a}	2.08^{ab}	70.70252	282.8101

Note: T3- seed and soil treatment; T4- soil and 30 DAT

treatment and at 30 DAT: Reimann's Sum (34.30548) and Area under Kinetic curve (137.2219).

Diversity indices were computed to determine the functional diversity of soil microbial communities in terms of carbon source utilization patterns. The corrected optical density for the 168 hr incubation period was used in the computation. Diversity indices of the different fertilizer management in 2023 DS, 2023 WS and 2024 DS were shown in Table 6 and 7. Significant differences were observed between the functional diversity indices of microbial communities from Day 1-Day 7 expressed as Shannon, Simpson's index and Evenness except for Day 1 (1/D). Diverse community and richness of species was observed using the functional indices which explains that plot applied with the use of 50 % RCM combined with NF has a capability to increase the microbial community available in the soil.

In 2023 Dry Season, plots applied with 50 % RCM + NF (T2, T3 and T4) had a significantly higher Shannon Index compared with the other treatments indicating higher microbial diversity. On the other hand, lowest Shannon Index was observed in Treatment 1 (RCM) and Treatment 6 (No Fertilizer) (Table 6). During the Dry Season of the following year (2024), plots treated with 15 g of NF plus 50 % RCM (T3) were observed to have the highest value (3.41) at Day 1 in terms of Shannon Index. On the other hand, plots with no fertilizer applied recorded the highest Shannon Index (3.38) at Day 7. However, plots with full fertilizer applied (T1) obtained the lowest (2.71) Shannon Index at Day 1 and at Day 7 (3.03) (Table 6). It is important to monitor the decreasing value of Shannon Index from plots with continuous synthetic fertilizer application. In agricultural systems, including rice fields, a Shannon Index value above 3 is often considered indicative of good microbial diversity (13, 14). In 2023 Wet Season, all the treatments recorded a Shannon Index of above 3.0 (Table 7).

The use of sole carbon source utilization patterns or community level physiological profiling in describing microbial functional communities offer a fast, highly reproducible technique that covers a wide spectrum of possible microbial specimens (15). However, the major disadvantages of using this method are: i) the result only represents the cultivable fraction of microbial communities; ii) fast growing organisms are favored; iii) bias to those organisms that can effectively utilize the available carbon sources; and iv) sensitivity to inoculum density.

Evaluation and Characterization of root system development

Root samples were collected after the crops' physiological maturity. Highest total root length (cm) was obtained in plots with 100 % RCM applied (Fig. 4). This was followed by plots with Treatment 4 (50 % RCM with NF applied 2x). Intermediate total root length was recorded in plants with no fertilizer.

On the other hand, highest total lateral root length (TLRL) per total nodal root length (TNRL) was recorded in plots applied with Treatment 4 (50 % RCM + NF (applied 2x)) while lowest record was obtained by T3 with 50 % RCM + NF applied

Table 6. Functional diversity index (Shannon H') of soil microbial communities based on 168-hr incubation period for 31 sole carbon substrates of Ecoplates. 2023 Dry Season and 2024 Dry Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

TREATMENT	2023 DS				2024 DS			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
T1. RCM	3.31^{b}	3.40^{b}	3.39^{bc}	3.21^{b}	2.71	3.01^{b}	2.99^{c}	3.03^{c}
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	3.35^{a}	3.42^{ab}	3.42^{ab}	3.23^{b}	2.95	3.04^{b}	3.11^{bc}	3.15^{bc}
T3. 50 % RCM + 15g NF (applied 2x)	3.36^{a}	3.42^{a}	3.43^{a}	3.43^{a}	3.41	3.11^{ab}	3.08^{bc}	3.06^{c}
T4. 50 % RCM + 15g NF (applied 2x)	3.36^{a}	3.41^{ab}	3.42^{a}	3.36^{a}	3.31	3.18^{ab}	3.20^{ab}	3.25^{ab}
T5. 50 % RCM + 20g NF (applied 3x)	3.30^{b}	3.37^{c}	3.38^{c}	3.40^{a}	3.30	3.15^{ab}	3.15^{abc}	3.18^{bc}
T6. No fertilizer	3.31^{b}	3.37^{c}	3.39^{c}	3.21^{b}	3.26	3.29^{a}	3.33^{a}	3.38^{a}

Note: T3- seed and soil treatment; T4- soil and 30 DAT

Table 7. Functional diversity index (Shannon H') of soil microbial communities based on 168-hr incubation period for 31 sole carbon substrates of Ecoplates. 2023 Wet Season, PhilRice CES, Maligaya, Science City of Muñoz, Nueva Ecija

TREATMENT	2023 WS			
	Day 1	Day 3	Day 4	Day 7
T1. RCM	3.31^{b}	3.40^{b}	3.39^{bc}	3.38^{a}
T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	3.35^{a}	3.42^{ab}	3.42^{ab}	3.39^{a}
T3. 50 % RCM + 15g NF (applied 2x)	3.36^{a}	3.42^{a}	3.43^{a}	3.33^{ab}
T4. 50 % RCM + 15g NF (applied 2x)	3.36^{a}	3.41^{ab}	3.42^{a}	3.23^{ab}
T5. 50 % RCM + 20g NF (applied 3x)	3.30^{b}	3.37^{c}	3.38^{c}	3.14^{b}
T6. No fertilizer	3.31^{b}	3.37^{c}	3.39^{c}	3.32^{ab}

Note: T3- seed and soil treatment; T4- soil and 30 DAT

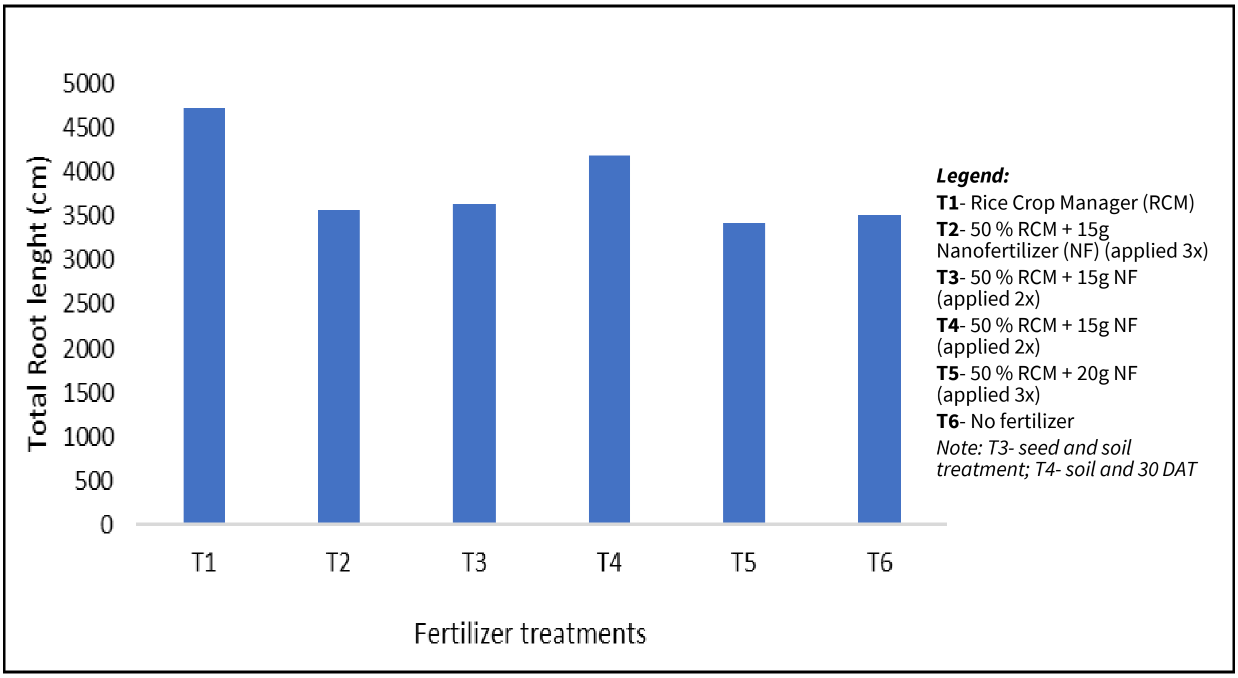

Fig. 4. Total root length of NSIC 222 at physiological maturity is influenced by fertilizer treatments.

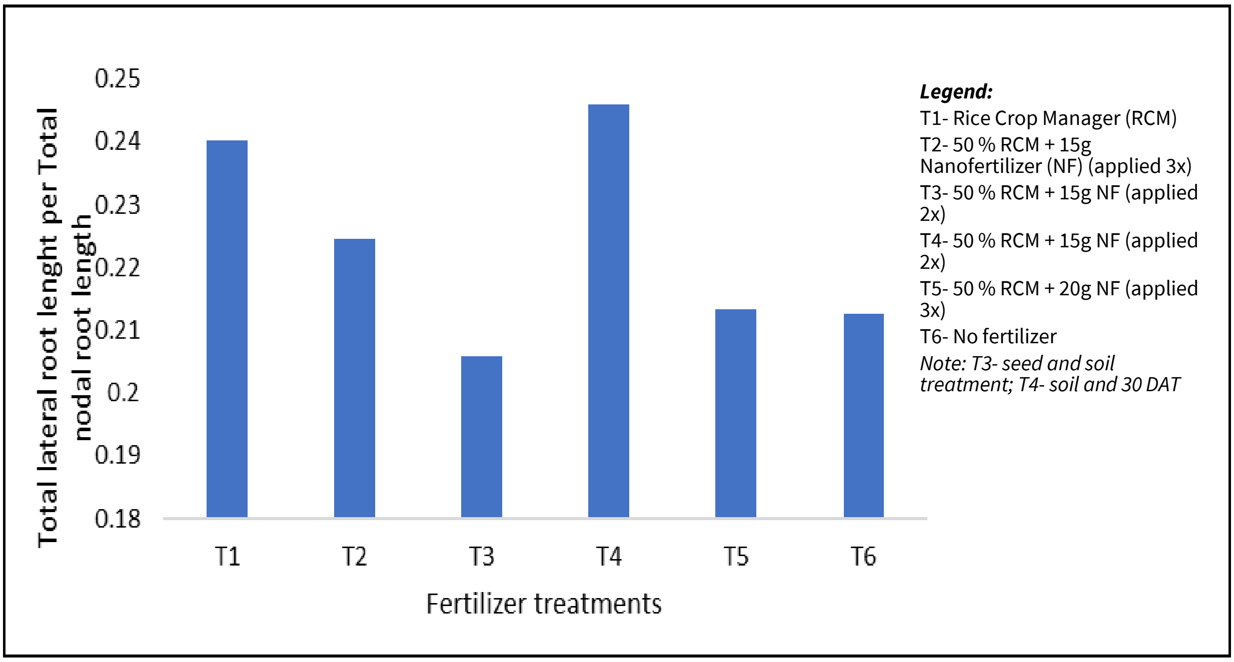

Fig. 5. Total lateral root length per total nodal root length of NSIC 222 at physiological maturity as influenced by fertilizer treatment.

twice (Fig. 5). Heaviest root dry weight and longest total root length per root dry weight was recorded in plots applied with 100 % RCM alone. However, data for TRL/RDW was comparable with the data obtained by T4 (50 % RCM + 15g NF) (Fig. 6). Generally, data on the roots including the total root length, root dry weight and total root length/ root dry weight were all obtained by plants treated with 100 % RCM followed by plots applied with 50 % RCM combined with NF. A 100 % application of the chemical fertilizer clearly showed that it allows the development of roots due to the presence of macronutrient applied during its growing period that resulted in the longer root length and develop more numbers of lateral roots. The integration of NPK fertilizer influence plant growth and development. The root biomass in the NPK was higher than the single fertilizer alone in improving root growth. It increased grain yield due to nitrogen use efficiency that improved root characteristics (16). However, soil health status of the area should also be monitored as continuous application of synthetic might affect the fertility of the soil in the area.

Treatment 1 (RCM) produced the highest length per root dry with 2299.58 which has no significant differences in Treatment 4 with 50 % RCM plus 15 g of NF applied twice (2298.19) (Fig. 7). Nano fertilizer serves as a soil conditioner that contributes to root system development. It contains living microorganisms that help in the development of root system and better seed germination (17).

Partial budget analysis

In 2022 Wet Season, highest gross income was obtained from Treatment 1 (RCM). On the other hand, highest net income was recorded in Treatment 6 (No fertilizer), PhP 104458.90; followed by Treatment 2 (15 g/ha NF combined with 50 % reduced inorganic fertilizer rate), PhP 102017.87. This is possibly due to the high cost of synthetic fertilizer in 2022. The indigenous

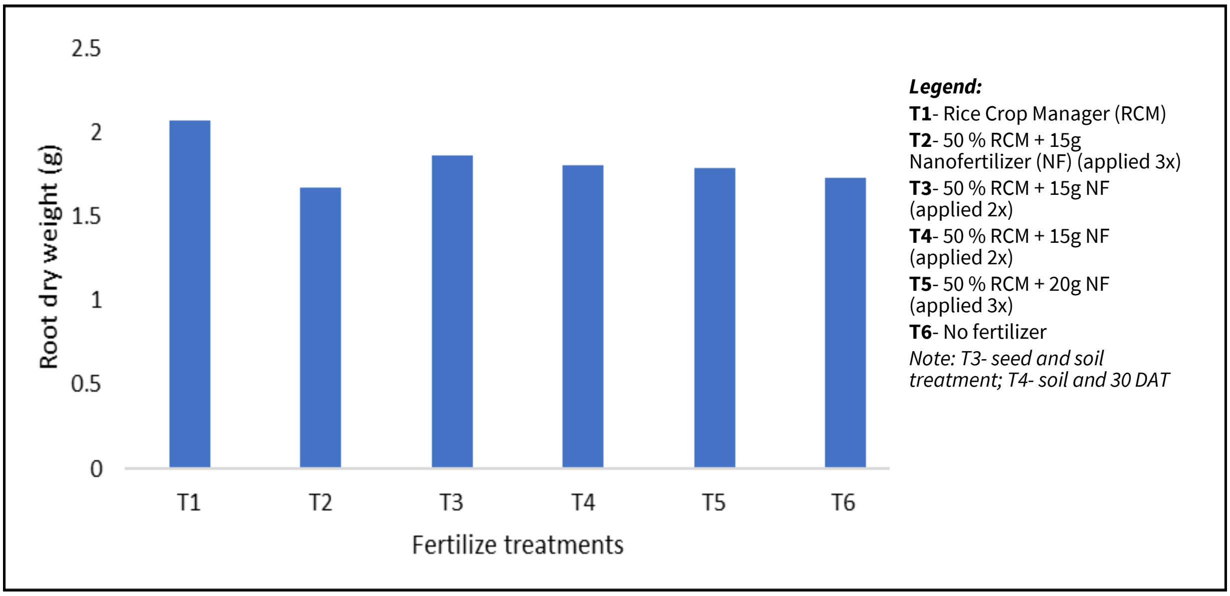

Fig. 6. Root dry weight of NSIC 222 at physiological maturity as influenced by various fertilizers.

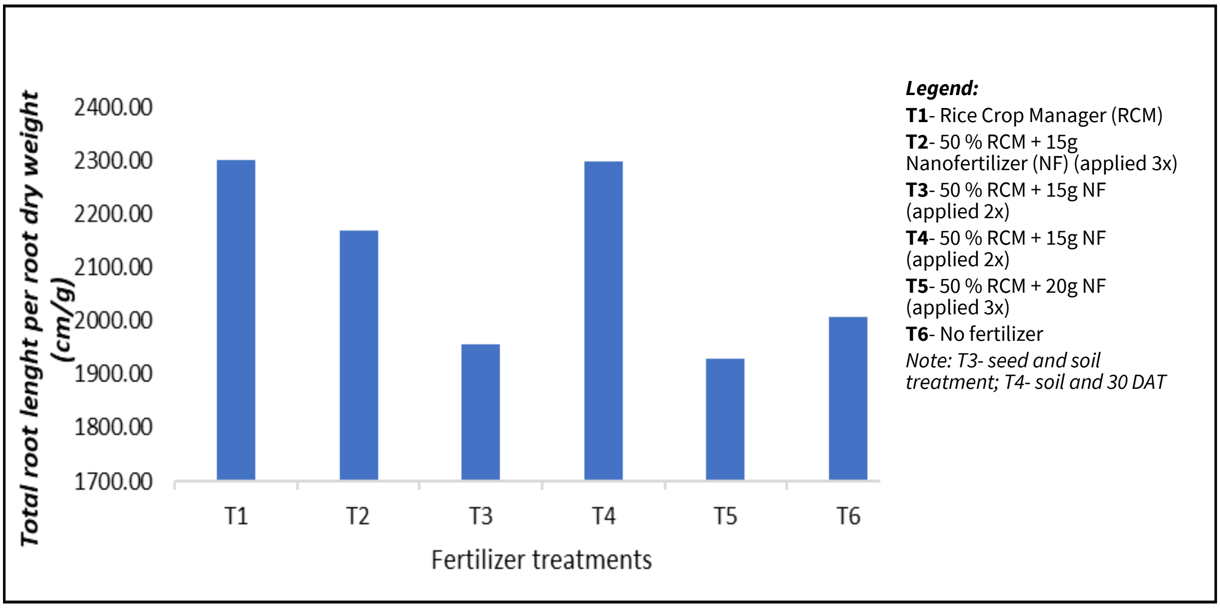

Fig. 7. Total root length per root dry weight of NSIC 222 at physiological maturity as influenced by fertilizer treatments.

nutrient supply in the soil is still enough that possibly contributed to the 5.9 tha^{-1} of Treatment 6. In Wet Season of the following year (2023), the 15 g/ha NF combined with 50 % reduced inorganic fertilizer rate (50 % RCM) applied during seed soaking, as soil treatment and at 30 days after transplanting (DAT) (T2) showed a higher grain yield with an added net income of PhP 13254.13 followed by T4 (PhP 12224.13).

Full inorganic fertilizer (100 % RCM) showed a higher grain yield and a higher gross income (PhP 152250.3) compared to other treatments in 2023 Dry Season. However, a higher cost of fertilizer input was also observed. The 15 g/ha NF combined with 50 % reduced inorganic fertilizer rate (50 % RCM) applied during soil treatment and at 30 days after transplanting (DAT) (T4) showed a higher grain yield next to the full dose of inorganic fertilizer with a net income of PhP 131979.97. On the other hand, lowest gross income was observed in the plots with no fertilizer. Added net income of Php 62494.17 was obtained from T4 relative to T6 (No Fertilizer). In 2024 Dry Season, full inorganic fertilizer (100 % RCM) showed a higher grain yield and a higher gross income (PhP 160413.50) compared to other treatments. However, a higher cost of fertilizer input was also observed. The 15 g/ha NF combined with 50 % RCM applied during soil treatment and at 30 days after transplanting (DAT) (T4) showed a higher grain yield next to the full dose of inorganic fertilizer with a net income of PhP 123023.63. On the other hand, lowest net income was observed in the plots with no fertilizer (Php 90480.00) (Table 8).

Supplementary data on soil microbial diversity analysis using next generation sequencing analysis

Based on the results of next generation sequencing, the dominant genus in Treatment 1 (RCM) belongs to *Acidobacteria* (acid-loving group of bacteria). This could be attributed to the continuous application of synthetic fertilizer in the plots which affect the pH of the soil. On the other hand, firmicutes, proteobacteria and chloroflexi, are the dominant microorganisms present in plots treated with 50 % RCM plus NF (T2 to T5). These groups of microorganisms are useful in biocontrol, bioremediation, nutrient transformation, organic matter degradation and nutrient transformation. Under no fertilizer treatment (T6), nitrospirae are the dominant microorganisms (Fig. 8).

Table 8. Partial budget analysis of rice production using different fertilizer treatments. 2022 Wet Season to 2024 Dry Season, PhilRice Central Experiment Station, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines

	TREATMENT (T)	GRAIN YIELD, kg/ha	GROSS INCOME, PhP	FERTILIZER COST, PhP	NET INCOME (NI), PhP	Added NI, Php
2022 Wet Season	T1. RCM	6,872	121569.89	20991.64	100578.24	(3880.66)
	T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	6,615	117013.69	14995.82	102017.87	(2441.03)
	T3. 50 % RCM + 15g NF (applied 2x)	6,439	113910.13	14995.82	98914.31	(5544.59)
	T4. 50 % RCM + 15g NF (applied 2x)	6,357	112463.32	14995.82	97467.50	(6991.40)
	T5. 50 % RCM + 20g NF (applied 3x)	6,268	110888.70	16495.82	94392.88	(10066.02)
	T6. No fertilizer	5,905	104458.90	0.00	104458.90	-
2023 Dry Season	T1. RCM	8370	152250.3	12711.154	139539.15	70053.35
	T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	7500	136425.00	10811.54	125613.47	56127.67
	T3. 50 % RCM + 15g NF (applied 2x)	7760	141154.40	10811.54	130342.87	60857.07
	T4. 50 % RCM + 15g NF (applied 2x)	7850	142791.50	10811.54	131979.97	62494.17
	T5. 50 % RCM + 20g NF (applied 3x)	7510	136606.90	12311.54	124295.37	54809.57
	T6. No fertilizer	3820	69485.80	0.00	69485.80	-
2023 Wet Season	T1. RCM	5350	110210.00	15991.75	94218.25	4608.25
	T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	5600	115360.00	12495.88	102864.13	13254.13
	T3. 50 % RCM + 15g NF (applied 2x)	5350	110210.00	12495.88	97714.13	8104.13
	T4. 50 % RCM + 15g NF (applied 2x)	5550	114330.00	12495.88	101834.13	12224.13
	T5. 50 % RCM + 20g NF (applied 3x)	4880	100528.00	13995.88	86532.13	(3077.87)
	T6. No fertilizer	4350	89610	0.00	89610	-
2024 Dry Season	T1. RCM	8510	160413.50	10874.38	149539.12	59059.12
	T2. 50 % RCM + 15g Nanofertilizer (NF) (applied 3x)	6890	129876.50	10999.87	118876.63	28396.63
	T3. 50 % RCM + 15g NF (applied 2x)	6880	129688.00	10999.87	118688.13	28208.13
	T4. 50 % RCM + 15g NF (applied 2x)	7110	134023.50	10999.87	123023.63	32543.63
	T5. 50 % RCM + 20g NF (applied 3x)	6080	114608.00	12499.87	102108.13	11628.13
	T6. No fertilizer	4800	90480.00	0.00	90480.00	-

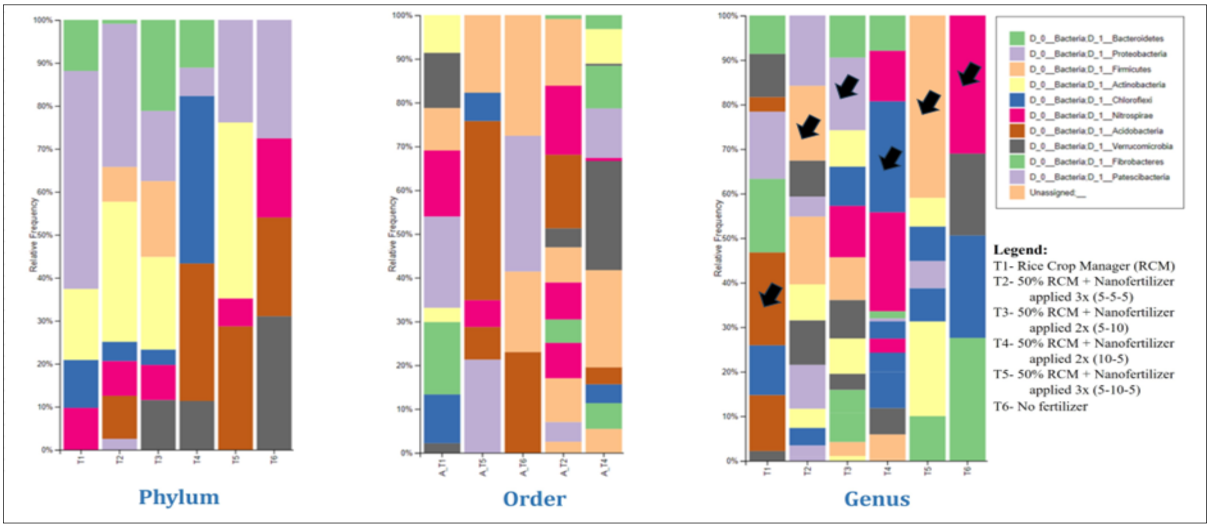

Fig. 8. Soil microbial diversity analysis is affected by different fertilizer treatments.

Conclusion

The study conducted across multiple cropping seasons (2022 Wet Season to 2024 Dry Season) demonstrates that integrating nano fertilizer with a 50 % reduced RCM (Recommended Crop Management) rate offers a promising alternative to full-dose inorganic fertilization. Despite external challenges like typhoon-induced lodging in the 2022 Wet Season, the combination of NF with reduced RCM rates consistently achieved yields comparable to full RCM treatments, showcasing its potential to maintain productivity with lower fertilizer inputs. This approach not only sustained crop yield but also enhanced soil microbial activity, as evidenced by higher AWCD, Riemann's Sum and Shannon Index values, indicating a more diverse and active soil microbial community. Additionally, while full RCM application led to the most robust root systems, the combination of reduced RCM with NF also promoted favorable root development, making it a viable option for reducing reliance on synthetic fertilizers.

Economically, the partial budget analysis revealed that although full RCM treatments produced the highest gross income, the high costs of synthetic fertilizers often reduced net profitability. In contrast, treatments combining NF with 50 % RCM consistently resulted in competitive yields and higher net incomes due to lower input costs. These findings suggest that this combined approach offers a sustainable and cost-effective strategy for maximizing agricultural productivity and profitability, while also potentially enhancing soil health and reducing environmental impact.

Recommendations

The promising results from the use of NF combined with reduced RCM rates warrant further investigation. Long-term studies should be conducted to assess the sustainability, cost-effectiveness and broader environmental impact of this approach.

Continuous monitoring of soil health indicators, such as the Shannon Index and AWCD, is crucial for assessing the long-term impacts of fertilizer management strategies. Specifically, attention should be paid to the decreasing Shannon Index in plots with continuous synthetic fertilizer application, as it may signal declining soil microbial diversity, which is critical for maintaining soil fertility.

While full RCM application maximizes yields, the high costs associated with synthetic fertilizers can reduce profitability. Farmers should evaluate current fertilizer prices and consider using reduced RCM rates combined with NF to optimize input costs and maintain profitability.

By adopting these recommendations, farmers can achieve sustainable crop production, optimize resource use and improve profitability, while also contributing to long-term soil health and environmental sustainability.

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Authors' contributions

JK carried out microbial studies, interpretation of data and drafted the manuscript. DA performed the statistical analysis and interpretation of data. SC participated in interpretation of data and involved in the manuscript drafting. RS carried out root system development studies and performed the interpretation of root analysis. All authors read and approved of the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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