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# Impact of Nitrogen Fertilization and Microbial Inoculants on Soil Microbial Biomass, Enzyme Activities and Carbon Respiration in DSR-rice Cultivation

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## ABSTRACT

**Background:** Rice is a staple crop grown globally, often requiring intensive nitrogen fertilization to achieve optimal yields. Excessive nitrogen use, however, can lead to environmental degradation and reduced soil health. Microbial inoculants, such as azospirillum, streptomyces and arbuscular mycorrhizal fungi (AMF), have shown potential in enhancing nutrient uptake and promoting sustainable agriculture.

**Methods:** This study evaluated the impact of three nitrogen application levels ($M_1$: 0 kg ha$^{-1}$, $M_2$: 40 kg ha$^{-1}$, $M_3$: 60 kg ha$^{-1}$ as the main plot and four microbial inoculation treatments ($S_1$: Control, $S_2$: Azospirillum (618 g ha$^{-1}$) + Streptomyces (618 g ha$^{-1}$), $S_3$: Consortium (1235 g ha$^{-1}$) and $S_4$: AMF (1235 g ha$^{-1}$) as the sub plot. A split-plot design (SPD) with three replications was employed to analyze their effects on soil and plant parameters in rice.

**Result:** The study revealed that the nitrogen levels and microbial inoculants significantly influenced soil and plant parameters in rice. The highest microbial biomass carbon (270.48 mg kg$^{-1}$), dehydrogenase activity (20.32 µg TPF g$^{-1}$ h$^{-1}$) and soil respiration (199.63 mg CO, kg$^{-1}$ day$^{-1}$) were observed with the consortium inoculant at 40 kg N ha$^{-1}$. AMF recorded maximum AMF colonization (56.20%) and enhanced microbial diversity (3.51 Shannon Index). Nitrogen application at 40 kg ha$^{-1}$ improved root length density (2.46 cm cm$^{-3}$), root biomass (18.23 g plant$^{-1}$) and root-to-shoot ratio (0.35). Soil organic carbon peaked at 0.70% with the consortium, while available N, P and K reached 279, 29 and 232 kg ha$^{-1}$, respectively, under 60 kg N ha$^{-1}$ and consortium. Interactions showed synergistic effects, optimizing soil and crop performance.

**Key words:** Arbuscular mycorrhizal fungi (AMF), Dehydrogenase activity (DHA), Microbial biomass carbon (MBC), Root length density (RLD), Soil organic carbon (SOC).

## INTRODUCTION

In India, rice is a staple crop, with the area under cultivation estimated at 47.6 million hectares in the fiscal year 2024 (Vajiram and Ravi, 2024). This represents approximately 29.6% of the global rice cultivation area, which stands at about 161 million hectares (Cordero *et al.,* 2020). India's rice production for the 2023-2024 period is projected at a record 137.83 million metric tons, accounting for 26% of global production (PJTSAU, 2024). The adoption of direct-seeded rice (DSR) has emerged as a sustainable strategy to address challenges related to water scarcity, energy costs and climate change. The transition to DSR demands effective soil and nutrient management strategies to sustain soil health and productivity under reduced water and intensive cropping conditions.

Soil microorganisms are crucial for maintaining soil biodiversity and play a significant role in the coordinated management of nutrients, which is essential for plant growth and development. Over the past few decades, the extensive application of chemical fertilizers has contributed to enhancing agricultural productivity, thus supporting national food self-sufficiency. However, this has come at a considerable environmental cost, affecting both ecosystem health and the sustainability of agricultural practices. The overuse of chemical fertilizers not only incurs high costs but also poses detrimental effects on soil fertility (Kalika-Singh *et al.,* 2022). In response to these challenges, the scientific community has focused on developing innovative and sustainable agricultural practices aimed at increasing crop yields, satisfying global food demands and mitigating environmental impacts (Comite *et al.,* 2021). Microbial inoculants are organisms that are introduced into an environment for a specific purpose, such as biocontrol or plant development and include bacteria, fungus and other microorganisms (Ramya *et al.,* 2019).

Microbial inoculants such as Azospirillum, Streptomyces, Arbuscular Mycorrhizal Fungi (AMF) and microbial consortia are effective in improving nutrient cycling, organic matter decomposition and overall soil

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microbial biomass. Azospirillum, a plant growth-promoting rhizobacterium, is renowned for its ability to fix atmospheric nitrogen and produce phytohormones, such as indole acetic acid (IAA). This enhances root proliferation, water uptake and nutrient acquisition, reduce nitrogen fertilizer requirements by 25-30% for rice cultivation (Reddy *et al.,* 2020). Streptomyces, a genus of actinobacteria, is vital for decomposing organic matter and producing secondary metabolites that suppress soil-borne pathogens. Its role in enhancing enzymatic activities, particularly dehydrogenase, urease and phosphatase, nutrient mineralization and improve soil structure, ultimately benefiting plant growth (Wang *et al.,* 2021). AMF establish symbiotic associations with plant roots, improving phosphorus solubilization and uptake a critical nutrient often limiting in rice systems. The use of microbial consortia, a synergistic blend of multiple beneficial microbes, has gained traction in sustainable agriculture. A consortium applied combines the benefits of Azospirillum, Streptomyces, AMF and other microbial species, significantly enhancing enzymatic activities and soil microbial biomass. This approach has been found to improve carbon respiration and nutrient cycling, contributing to higher crop productivity and better soil health (Kumutha *et al.,* 2023).

Nitrogen (N) fertilization plays a pivotal role in ensuring high yields in rice systems, but excessive and imbalanced use can degrade soil health by reducing microbial diversity, suppressing enzymatic activities and increasing nitrate leaching. Thus, integrating microbial inoculants with balanced nitrogen fertilization has been explored as a promising approach to enhance soil fertility and promote sustainable agricultural practices. This study evaluated the effects of different nitrogen fertilization levels and microbial inoculation treatments on soil microbial biomass, enzyme activities and carbon respiration, the research aimed to explore sustainable strategies for enhancing soil fertility and rice yield in direct-seeded rice systems.

## MATERIALS AND METHODS

The field experiment was conducted during the *kharif* season of 2024 at the research farm of the Department of Agronomy, School of Agriculture, Lovely Professional University (LPU), Punjab. The research site is geographically located at a latitude of 31°24′N and a longitude of 75°69′W, with an altitude of 245 m above mean sea level. The study utilized a split-plot design (SPD) with three replications. The experiment comprised two factors. The main plot involved three nitrogen application levels: $M_1$: 0 kg ha$^{-1}$, $M_2$: 40 kg ha$^{-1}$ and $M_3$: 60 kg ha$^{-1}$. The subplot included four microbial inoculation treatments: $S_1$: Control (no microbial inoculation), $S_2$: Azospirillum (618 g ha$^{-1}$) + Streptomyces (618 g ha$^{-1}$), $S_3$: Consortium (1235 g ha$^{-1}$) and $S_4$: Arbuscular mycorrhizal fungi (AMF) (1235 g ha$^{-1}$). Each experimental plot measured 20 m$^2$, with dimensions of 5 m in width and 4 m in length. The crop spacing was maintained at 20 cm between rows and 15 cm between plants. Based on preliminary soil analysis, all plots were uniformly supplied with phosphorus and potassium at a rate of 30 kg ha$^{-1}$ each. Recommended dose of NPK was applied according to the treatment specifications. The sources of fertilizers were urea (46% N), single super phosphate (16% $P_2O_5$) and muriate of potash (60% $K_2O$). A half dose of nitrogen, along with the full doses of phosphorus and potassium, was applied as a basal dose at the time of planting. The remaining half dose of nitrogen was applied in two equal splits at the active tillering and panicle initiation stages. The microbial inoculants were applied using a seed treatment technique. Seeds were treated with the respective microbial inoculants as per the treatment allocations before sowing. The rice variety ‘Pusa Basmati 1509’ was used in the study. The crop was sown on June 12th and harvested on October 14th, 2024. Weeds in the experimental plots were managed by application of Pendimethalin 30 EC @1.0 kg a.i. ha$^{-1}$ at 3 days after sowing as pre-emergent and Bispyribac sodium 10% SL @ 250 ml ha$^{-1}$ was sprayed at 20 days after sowing. Meteorological data, including rainfall and temperature, were recorded throughout the experiment. The total rainfall during the growing season was 328.2 mm, with the highest rainfall occurring in June. The maximum temperature recorded was 46.8°C in June, while the minimum temperature was 18.4°C in October. Soil samples (0-20 cm depth) were collected from the experimental site before sowing and after harvesting and analysed for physicochemical and biological properties.

Microbial biomass carbon (MBC) was measured using the fumigation-extraction method, expressed in mg kg$^{-1}$ soil. Dehydrogenase Activity was assessed through the reduction of TTC to TPF, reported in μg TPF g$^{-1}$ h$^{-1}$. Soil respiration was quantified by measuring $CO_2$ evolution over 24 hours, expressed in mg $CO_2$ kg$^{-1}$ day$^{-1}$. AMF Colonization was determined by staining roots and quantifying root colonization percentage. Nitrogen Fixing Bacteria were quantified using CFU counts from serial dilution and plating. Microbial Diversity was evaluated using the Shannon Diversity Index, derived from species data *via* molecular or culture methods.

### Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). The differences among the treatment means were separated using the least significant difference (LSD) test at a significance level of $p \leq 0.05$. All statistical analyses were conducted using Origin Pro software (version 10.1.0.178).

## RESULTS AND DISCUSSION

### Effect of nitrogen application levels and microbial inoculation on soil biological properties

Nitrogen application levels and microbial inoculation had significant effects on microbial biomass carbon (MBC), dehydrogenase activity (DHA) and soil respiration (SR).

The application of 40 kg N ha$^{-1}$ (M$_2$) produced the highest MBC (242.12 mg kg$^{-1}$), DHA (17.54 µg TPF g$^{-1}$ h$^{-1}$) and SR (183.64 mg CO,  kg$^{-1}$ day$^{-1}$), indicating an optimal nitrogen dose for enhancing microbial activity (Table 1). Among microbial inoculations, the consortium treatment (S$_3$) recorded the highest values for MBC (260.11 mg kg$^{-1}$), DHA (19.44 µg TPF g$^{-1}$ h$^{-1}$) and SR (193.41 mg CO, kg$^{-1}$ day$^{-1}$). This suggests that the synergistic effect of microbial consortia improved soil biological processes. These results align with previous studies, where integrated microbial treatments demonstrated positive impacts on soil microbial activities and nutrient cycling (Wang *et al.,* 2021).

**Interaction effects of nitrogen application levels and microbial inoculation on soil biological properties**

The interaction between nitrogen levels and microbial inoculation was significant for all parameters (Table 2). The combination of 40 kg N ha$^{-1}$ (M$_2$) and Consortium (S$_3$) yielded the highest MBC (270.48 mg kg$^{-1}$), DHA (20.15 µg TPF g$^{-1}$ h$^{-1}$) and SR (199.63 mg CO, kg$^{-1}$ day$^{-1}$), reflecting the potential of integrating moderate nitrogen levels with microbial inoculation for improved soil health. Lower activity was observed in zero nitrogen (M$_1$) and control treatments (S$_1$), underscoring the need for external inputs to stimulate microbial functioning. These findings collaborate earlier

**Table 1:** Effect of nitrogen application levels and microbial inoculation on microbial biomass carbon, dehydrogenase activity and soil respiration.

| Treatments | Microbial biomass carbon (mg kg$^{-1}$) | Dehydrogenase activity (ug TPF g$^{-1}$ h$^{-1}$) | Soil respiration (mg CO$_2$ kg$^{-1}$ day$^{-1}$) |
|---|---|---|---|
| **Nitrogen application levels** | | | |
| M$_1$: (N-0 kg ha$^{-1}$) | 221.65 | 15.50 | 172.50 |
| M$_2$: (N-40 kg ha$^{-1}$) | 242.12 | 17.54 | 183.64 |
| M$_3$: (N-60 kg ha$^{-1}$) | 232.66 | 17.01 | 175.84 |
| **Microbial inoculation** | | | |
| S$_1$: (Control) | 190.32 | 13.25 | 154.22 |
| S$_2$: (Azospirillum (618 g ha$^{-1}$) + Streptomyces's (618 g ha$^{-1}$) | 231.58 | 16.23 | 174.36 |
| S$_3$: (Consortium (1235 g ha$^{-1}$) | 260.11 | 19.44 | 193.41 |
| S$_4$: (AMF (1235 g ha$^{-1}$) | 246.39 | 18.09 | 186.20 |
| SD | 28.17 | 3.06 | 17.11 |
| S. Em (±) | 4.69 | 0.51 | 2.85 |

**Table 2:** Interaction effects of nitrogen application levels and microbial inoculation on microbial biomass carbon, dehydrogenase activity and soil respiration.

| Treatments combination | | Microbial biomass carbon (mg kg$^{-1}$) | Dehydrogenase activity (ug TPF g$^{-1}$ h$^{-1}$) | Soil respiration (mg CO$_2$ kg$^{-1}$ day$^{-1}$) |
|---|---|---|---|---|
| M$_1$ | S$_1$ | 181.32 | 12.33 | 150.32 |
| | S$_2$ | 222.22 | 16.24 | 169.87 |
| | S$_3$ | 251.46 | 18.98 | 190.14 |
| | S$_4$ | 231.89 | 16.54 | 180.87 |
| M$_2$ | S$_1$ | 199.42 | 14.33 | 160.22 |
| | S$_2$ | 240.63 | 16.52 | 180.45 |
| | S$_3$ | 270.48 | 20.15 | 199.63 |
| | S$_4$ | 259.31 | 19.34 | 194.78 |
| M$_3$ | S$_1$ | 189.41 | 13.20 | 153.33 |
| | S$_2$ | 230.22 | 16.47 | 174.82 |
| | S$_3$ | 259.64 | 20.32 | 19.43 |
| | S$_4$ | 249.30 | 18.86 | 185.01 |
| LSD (p=0.05) | | 3.24 | 3.16 | 13.74 |
| Main plot | | <0.0001 | <0.0001 | <0.0001 |
| Sub plot | | 7.81164E-4 | 0.85 | 0.99 |
| Interaction | | <0.0001 | <0.0001 | <0.0001 |

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05< .< 0.1 < ° < 1 research emphasizing the role of microbial consortia in enhancing soil fertility and productivity under sustainable management practices (Zhang *et al.,* 2020).

### Effects of nitrogen application levels and microbial inoculants on amf colonization, nitrogen fixing bacteria and microbial diversity

The nitrogen application levels and microbial inoculants significantly influenced arbuscular mycorrhizal fungi (AMF) colonization, nitrogen-fixing bacteria (NFB) and microbial diversity (Shannon Index) in rice soils. The highest AMF colonization (47.91% root colonization) and microbial diversity (3.37) were observed with 40 kg N ha$^{-1}$ (M$_2$), while 60 kg N ha$^{-1}$ (M$_3$) had slightly lower values (43.16% and 3.25, respectively) (Table 3). Among microbial inoculants, AMF treatment (S4) demonstrated the highest AMF colonization (56.20%) and microbial diversity (3.46), whereas the consortium treatment (S$_3$) recorded the highest NFB (3.13 CFU g$^{-1}$ soil). These findings indicate that moderate nitrogen levels coupled with efficient microbial inoculants enhance soil microbial attributes, consistent with studies demonstrating the positive interaction between nitrogen input and microbial amendments on soil health (Singh *et al.,* 2022).

### Interaction effects of nitrogen application levels and microbial inoculation on amf colonization, nitrogen fixing bacteria and microbial diversity

The interaction effects between nitrogen levels and microbial inoculants were not statistically significant for

**Table 3:** Effects of nitrogen application levels and microbial inoculants on amf colonization, nitrogen fixing bacteria and microbial diversity in rice.

| Treatments | AMF colonization (% Root colonization) | Nitrogen fixing bacteria (CFU g$^{-1}$ soil) | Microbial diversity (Shannon index) |
|---|---|---|---|
| **Nitrogen application levels** | | | |
| M$_1$: (N-0 kg ha$^{-1}$) | 37.75 | 2.40 | 3.21 |
| M$_2$: (N-40 kg ha$^{-1}$) | 47.91 | 2.52 | 3.37 |
| M$_3$: (N-60 kg ha$^{-1}$) | 43.16 | 2.48 | 3.25 |
| **Microbial inoculation** | | | |
| S$_1$: (Control) | 27.63 | 1.34 | 2.97 |
| S$_2$: (Azospirillum (618 g ha$^{-1}$) + Streptomyces's (618 g ha$^{-1}$) | 40.74 | 2.8 | 3.30 |
| S$_3$: (Consortium (1235 g ha$^{-1}$) | 47.13 | 3.13 | 3.51 |
| S$_4$: (AMF (1235 g ha$^{-1}$) | 56.20 | 2.61 | 3.46 |
| SD | 11.92 | 0.69 | 0.26 |
| S. Em (±) | 1.98 | 0.11 | 0.04 |

**Table 4:** Interaction effects of nitrogen levels and microbial inoculants on AMF colonization, nitrogen fixing bacteria and microbial diversity in rice.

| Treatments combination | | AMF colonization (% Root colonization) | Nitrogen fixing bacteria (CFU g$^{-1}$ soil) | Microbial diversity (Shannon index) |
|---|---|---|---|---|
| M$_1$ | S$_1$ | 24.12 | 1.3 | 2.87 |
| | S$_2$ | 36.32 | 2.8 | 3.26 |
| | S$_3$ | 40.12 | 3.03 | 3.51 |
| | S$_4$ | 50.33 | 2.55 | 3.33 |
| M$_2$ | S$_1$ | 30.25 | 1.43 | 3.01 |
| | S$_2$ | 46.59 | 2.90 | 3.46 |
| | S$_3$ | 54.87 | 3.13 | 3.62 |
| | S$_4$ | 60.32 | 2.67 | 3.55 |
| M$_3$ | S$_1$ | 28.30 | 1.32 | 2.94 |
| | S$_2$ | 39.87 | 2.76 | 3.32 |
| | S$_3$ | 46.58 | 3.26 | 3.43 |
| | S$_4$ | 58.99 | 2.79 | 3.45 |
| LSD (p=0.05) | | 6.69 | 0.17 | 0.17 |
| Main plot | | <0.0001 | 0.03 | 0.001 |
| Sub plot | | <0.0001 | <0.0001 | <0.0001 |
| Interaction | | 0.48 | 0.089 | 0.82 |

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05< .< 0.1 < ° < 1.

AMF colonization and microbial diversity but showed notable trends (Table 4). The combination of 40 kg N ha$^{-1}$ ($M_2$) and AMF ($S_4$) achieved the highest AMF colonization (60.32%) and microbial diversity (3.55). NFB was highest (3.26 CFU g$^{-1}$ soil) in the 60 kg N ha$^{-1}$ ($M_3$) and Consortium ($S_3$) treatment. Control treatments consistently exhibited the lowest values for all parameters, underscoring the essential role of microbial inoculants in enhancing microbial functions. These results align with previous findings emphasizing the role of bioinoculants in improving microbial populations and diversity under sustainable nitrogen management (Zhao *et al.,* 2021).

### Effect of nitrogen levels and microbial inoculants on root traits in rice

The data in Table 5 demonstrates that nitrogen levels significantly influenced root traits in rice. The application of 40 kg ha$^{-1}$ nitrogen (M2) achieved the highest values for root length density (2.32 $cm/cm^3$), root biomass (16.55 g plant$^{-1}$), root volume (21.50 $cm^3$) and root-to-shoot ratio (0.32). These values were superior to those of 0 kg ha$^{-1}$ nitrogen ($M_1$), which recorded the lowest values for root length density (1.75 cm cm$^{-3}$), root biomass (14.26 g plant$^{-1}$), root volume (18.00 $cm^3$) and root-to-shoot ratio (0.28). Interestingly, increasing nitrogen application to 60 kg ha$^{-1}$

**Table 5:** Effect of nitrogen levels and microbial inoculants on root length density, root biomass, root volume and root to shoot ratio in rice.

| Treatments | Root length density (cm cm$^{-3}$) | Root biomass (g plant$^{-1}$) | Root volume ($cm^3$) | Root to shoot ratio |
|---|---|---|---|---|
| **Nitrogen application levels** | | | | |
| $M_1$: (N-0 kg ha$^{-1}$) | 1.75 | 14.26 | 18.00 | 0.28 |
| $M_2$: (N-40 kg ha$^{-1}$) | 2.32 | 16.55 | 21.50 | 0.32 |
| $M_3$: (N-60 kg ha$^{-1}$) | 2.07 | 16.02 | 19.16 | 0.31 |
| **Microbial inoculation** | | | | |
| $S_1$: (Control) | 1.32 | 12.86 | 15.47 | 0.27 |
| $S_2$: (Azospirillum (618 g ha$^{-1}$) + Streptomyces's (618 g ha$^{-1}$) | 2.13 | 16.13 | 20.22 | 0.30 |
| $S_3$: (Consortium (1235 g ha$^{-1}$) | 2.46 | 16.98 | 22.36 | 0.32 |
| $S_4$: (AMF (1235 g ha$^{-1}$) | 2.26 | 16.46 | 20.69 | 0.31 |
| SD | 0.50 | 2.10 | 3.62 | 0.04 |
| S. Em (±) | 0.08 | 0.35 | 0.60 | 0.006 |

**Table 6:** Interaction effects of nitrogen levels and microbial inoculants on root length density, root biomass, root volume and root to shoot ratio in rice.

| Treatments combination | | Root length density (cm cm$^{-3}$) | Root biomass (g plant$^{-1}$) | Root volume ($cm^3$) | Root to shoot ratio |
|---|---|---|---|---|---|
| $M_1$ | $S_1$ | 1.2 | 12.10 | 15.47 | 0.25 |
| | $S_2$ | 1.87 | 14.89 | 18.44 | 0.28 |
| | $S_3$ | 2.16 | 15.14 | 20.69 | 0.32 |
| | $S_4$ | 1.96 | 14.99 | 19.87 | 0.29 |
| $M_2$ | $S_1$ | 1.50 | 13.54 | 16.55 | 0.27 |
| | $S_2$ | 2.43 | 17.01 | 22.47 | 0.32 |
| | $S_3$ | 2.87 | 18.23 | 25.47 | 0.35 |
| | $S_4$ | 2.66 | 17.55 | 23.69 | 0.34 |
| $M_3$ | $S_1$ | 1.32 | 13.02 | 14.01 | 0.29 |
| | $S_2$ | 2.27 | 16.50 | 20.63 | 0.30 |
| | $S_3$ | 2.59 | 17.68 | 22.58 | 0.33 |
| | $S_4$ | 2.30 | 17.00 | 20.69 | 0.32 |
| LSD (p=0.05) | | 0.17 | 0.69 | 3.31 | 0.05 |
| Main plot | | <0.0001 | <0.0001 | 7.44508E-A | 0.03 |
| Sub plot | | <0.0001 | <0.0001 | <0.0001 | 0.02 |
| Interaction | | 0.02 | 0.81 | 0.57 | 0.97 |

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05< .< 0.1 < ° < 1

($M_3$) did not result in further improvements and even showed a slight decline in parameters such as root length density (2.07 cm cm$^{-3}$) and root volume (19.16 cm$^3$). This suggests that moderate nitrogen application (40 kg ha$^{-1}$) optimally supports root growth, aligning with reports of diminishing returns with higher nitrogen levels (Smith *et al.,* 2020).

Microbial inoculants also significantly enhanced root traits. The consortium treatment ($S_3$) outperformed all other treatments, recording the highest root length density (2.46 cm cm$^{-3}$), root biomass (16.98 g plant$^{-1}$), root volume (22.36 cm$^3$) and root-to-shoot ratio (0.32). The inoculant combination of Azospirillum and Streptomyces ($S_2$) and AMF ($S_4$) also improved root traits, with root biomass reaching 16.13 g plant$^{-1}$ and 16.46 g plant$^{-1}$, respectively, compared to the uninoculated control ($S_1$), which exhibited the lowest values: root length density (1.32 cm cm$^{-3}$), root biomass (12.86 g plant$^{-1}$), root volume (15.47 cm$^3$) and root-to-shoot ratio (0.27). These results align with findings by Sharma *et al.,* (2021), observed improvement highlights the ability of microbial inoculants to promote nutrient uptake and root architecture.

### Interaction effects of nitrogen levels and microbial inoculants on root traits in rice

The interaction effects presented in Table 6 further illustrate the combined influence of nitrogen levels and microbial inoculants. The combination of $M_2$ nitrogen level and Consortium ($S_3$) resulted in the highest values for all parameters, including root length density (2.87 cm cm$^{-3}$), root biomass (18.23 g plant$^{-1}$), root volume (25.47 cm$^3$) and root-to-shoot ratio (0.35). This synergistic effect underscores the importance of moderate nitrogen application coupled with a robust microbial inoculant in optimizing root development. In contrast, the interaction of M3 nitrogen level and uninoculated control ($S_1$) exhibited the lowest values: root length density (1.32 cm cm$^{-3}$), root biomass (13.02 g plant$^{-1}$), root volume (14.01 cm$^3$) and root-to-shoot ratio (0.29). This finding reinforces the limited efficacy of high nitrogen levels in the absence of microbial enhancement. Notably, under $M_1$ nitrogen level, the consortium ($S_3$) treatment still delivered considerable improvements, achieving root length density of 2.16 cm cm$^{-3}$, root biomass of 15.14 g plant$^{-1}$ and root volume of 20.69 cm$^3$, suggesting microbial inoculants can partially mitigate nitrogen limitations. Sharma *et al.* (2021) similarly reported that integrating moderate nitrogen levels with effective microbial inoculants, such as consortium, maximizes root development, enhancing water and nutrient acquisition.

### Influence of nitrogen levels and microbial inoculants on soil pH, electrical conductivity and soil organic carbon

The data presented in Table 7 highlights the effects of nitrogen application levels and microbial inoculants on soil pH, electrical conductivity (EC) and soil organic carbon (SOC). Among the nitrogen treatments, $M_2$ (N-40 kg ha$^{-1}$) showed the most balanced soil pH (7.40), EC (0.33 dS m$^{-1}$) and SOC (0.69%), indicating its optimal influence on soil health. In terms of microbial inoculants, the consortium ($S_3$) performed best, recording the highest SOC (0.70%) and balanced pH (7.52). The control treatment ($S_1$) consistently recorded the lowest values across all parameters, with SOC at 0.60%, pH at 7.32 and EC at 0.31 dS m$^{-1}$. These findings align with the study by Sharma *et al.,* (2021), emphasizing the role of microbial inoculants in improving soil health indicators.

### Interaction effects of nitrogen levels and microbial inoculants on soil pH, electrical conductivity and soil organic carbon

The interaction data in Table 8 shows that the combination of $M_2$ nitrogen level with consortium ($S_3$) resulted in the

**Table 7:** Influence of nitrogen levels and microbial inoculants on soil pH, electrical conductivity (EC) and soil organic carbon.

| Treatments | Soil pH | Soil EC (dS m$^{-1}$) | Soil organic carbon (%) |
|---|---|---|---|
| **Nitrogen application levels** | | | |
| $M_1$: (N-0 kg ha$^{-1}$) | 7.51 | 0.28 | 0.63 |
| $M_2$: (N-40 kg ha$^{-1}$) | 7.40 | 0.33 | 0.69 |
| $M_3$: (N-60 kg ha$^{-1}$) | 7.52 | 0.38 | 0.66 |
| **Microbial inoculation** | | | |
| $S_1$: (Control) | 7.32 | 0.31 | 0.60 |
| $S_2$: (Azospirillum (618 g ha$^{-1}$) + Streptomyces's (618 g ha$^{-1}$) | 7.46 | 0.33 | 0.67 |
| $S_3$: (Consortium (1235 g ha$^{-1}$) | 7.52 | 0.35 | 0.70 |
| $S_4$: (AMF (1235 g ha$^{-1}$) | 7.47 | 0.33 | 0.68 |
| SD | 0.12 | 0.045 | 0.045 |
| S. Em (±) | 0.02 | 0.007 | 0.007 |

**Table 8:** Interaction effects of nitrogen levels and microbial inoculants on soil pH, electrical conductivity (EC) and soil organic carbon.

| Treatments combination | | Soil pH | Soil EC (dS m$^{-1}$) | Soil organic carbon (%) |
|---|---|---|---|---|
| $M_1$ | $S_1$ | 7.40 | 0.26 | 0.59 |
| | $S_2$ | 7.50 | 0.28 | 0.63 |
| | $S_3$ | 7.61 | 0.29 | 0.66 |
| | $S_4$ | 7.52 | 0.27 | 0.64 |
| $M_2$ | $S_1$ | 7.32 | 0.31 | 0.61 |
| | $S_2$ | 7.4 | 0.33 | 0.69 |
| | $S_3$ | 7.52 | 0.35 | 0.73 |
| | $S_4$ | 7.47 | 0.32 | 0.71 |
| $M_3$ | $S_1$ | 7.22 | 0.36 | 0.60 |
| | $S_2$ | 7.36 | 0.37 | 0.67 |
| | $S_3$ | 7.48 | 0.39 | 0.70 |
| | $S_4$ | 7.31 | 0.38 | 0.68 |
| LSD ($p$=0.05) | | 0.13 | 2.57 | 2.57 |
| Main plot | | <0.0001 | <0.0001 | <0.0001 |
| Sub plot | | 4.09661E-4 | 0.001 | <0.0001 |
| Interaction | | 0.99 | 0.87 | 0.12 |

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05< .< 0.1 < ° < 1.

**Table 9:** Effect of nitrogen levels and microbial inoculants on available N, P and K in soil.

| Treatments | Available N (kg ha$^{-1}$) | Available P (kg ha$^{-1}$) | Available K (kg ha$^{-1}$) |
|---|---|---|---|
| **Nitrogen application levels** | | | |
| $M_1$: (N-0 kg ha$^{-1}$) | 211 | 15 | 188 |
| $M_2$: (N-40 kg ha$^{-1}$) | 261 | 21 | 211 |
| $M_3$: (N-60 kg ha$^{-1}$) | 276 | 27 | 228 |
| **Microbial inoculation** | | | |
| $S_1$: (Control) | 241 | 19 | 202 |
| $S_2$: (Azospirillum (618 g ha$^{-1}$) + Streptomyces's (618 g ha$^{-1}$) | 250 | 22 | 209 |
| $S_3$: (Consortium (1235 g ha$^{-1}$) | 254 | 23 | 214 |
| $S_4$: (AMF (1235 g ha$^{-1}$) | 251 | 21 | 211 |
| SD | 28.81 | 5.36 | 17.34 |
| S. Em (±) | 4.80 | 0.89 | 2.89 |

**Table 10:** Interaction effects of nitrogen levels and microbial inoculants on available N, P and K in soil.

| Treatments combination | | Available N (kg ha$^{-1}$) | Available P (kg ha$^{-1}$) | Available K (kg ha$^{-1}$) |
|---|---|---|---|---|
| $M_1$ | $S_1$ | 201 | 13 | 182 |
| | $S_2$ | 211 | 16 | 187 |
| | $S_3$ | 216 | 17 | 192 |
| | $S_4$ | 213 | 15 | 190 |
| $M_2$ | $S_1$ | 251 | 19 | 202 |
| | $S_2$ | 261 | 21 | 212 |
| | $S_3$ | 266 | 23 | 217 |
| | $S_4$ | 263 | 22 | 214 |
| $M_3$ | $S_1$ | 271 | 26 | 222 |
| | $S_2$ | 276 | 28 | 227 |
| | $S_3$ | 279 | 29 | 232 |
| | $S_4$ | 277 | 27 | 230 |
| LSD (p=0.05) | | 2.60 | 2.12 | 3.58 |
| Main plot | | <0.0001 | <0.0001 | <0.0001 |
| Sub plot | | <0.0001 | <0.0001 | <0.0001 |
| Interaction | | 0.01 | 0.74 | 0.31 |

Signification codes: 0 < *** < 0.001 < ** < 0.01 < * < 0.05< .< 0.1 < ° < 1.

highest SOC (0.73%) and optimal EC (0.35 dS m$^{-1}$). Similarly, $M_2$ with AMF ($S_4$) recorded balanced pH (7.47) and SOC (0.71%). The combination of $M_1$ nitrogen level with the control treatment ($S_1$) yielded the lowest SOC (0.59%) and pH (7.40), further underscoring the importance of microbial inoculation in enhancing soil properties.

### Effect of nitrogen levels and microbial inoculants on available nitrogen, phosphorus and potassium in soil

The results in Table 9 demonstrate that nitrogen application significantly influenced the availability of nitrogen (N), phosphorus (P) and potassium (K) in soil. The highest values for all nutrients were observed in the $M_3$ (N-60 kg ha$^{-1}$) treatment, with available N at 276 kg ha$^{-1}$, P at 27 kg ha$^{-1}$ and K at 228 kg ha$^{-1}$. Among microbial inoculants, the Consortium ($S_3$) outperformed others, with the highest available N (254 kg ha$^{-1}$), P (23 kg ha$^{-1}$) and K (214 kg ha$^{-1}$). The control treatment ($S_1$) consistently recorded the lowest nutrient availability, highlighting the efficacy of microbial inoculants in nutrient cycling. These results are supported by Smith *et al.* (2020), who observed similar trends in nutrient availability with microbial inoculant applications.

### Interaction effects of nitrogen levels and microbial inoculants on available nitrogen, phosphorus and potassium in soil

The interaction data in Table 10 indicates that the combination of $M_3$ nitrogen level with consortium ($S_3$) resulted in the highest nutrient availability: available N at 279 kg ha$^{-1}$, P at 29 kg ha$^{-1}$ and K at 232 kg ha$^{-1}$. This highlights the synergistic effect of higher nitrogen application and microbial inoculants on nutrient availability. In contrast, the combination of $M_1$ nitrogen level with the control treatment ($S_1$) yielded the lowest values, with available N at 201 kg ha$^{-1}$, P at 13 kg ha$^{-1}$ and K at 182 kg ha$^{-1}$.

## CONCLUSION

The study reveals that moderate nitrogen application (40 kg ha$^{-1}$, $M_2$) and microbial inoculation, particularly the consortium treatment ($S_3$), significantly enhance soil biological properties, root traits and nutrient availability. The $M_2$ + $S_3$ combination achieved the highest microbial biomass carbon (270.48 mg kg$^{-1}$), dehydrogenase activity (20.15 µg TPF g$^{-1}$ h$^{-1}$), root length density (2.87 cm cm$^{-3}$) and available nitrogen (279 kg ha$^{-1}$), emphasizing the synergy between moderate nitrogen and effective microbial inoculants for sustainable soil and crop management.

### Conflict of interest

The authors declare no conflict of interest.

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