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Differential Response of Rhizosphere Microbial Diversity and Activities to System of Rice Intensification and Conventional Cultivation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice holds a vital position among staple crops worldwide, serving as a primary source of essential nutrition for a significant portion of the global population. Rice production is currently plagued by several problems causing a decline in crop yields. System of Rice Intensification (SRI) is a set of farming practices designed to improve the productivity and sustainability of rice cultivation. One of the reasons for the sustainability of SRI has been attributed to enhanced below-ground soil microbial processes around the SRI plant root system. This study assessed the effect of SRI and Normal Transplanting (NTP) cultivation methods on rhizospheric soil microbial populations, phyto-hormones, soil enzyme activities under four different nitrogen (N) treatments under a station trial and in on-farms experiments farmer's. Rhizosphere soils and root samples of SRI exhibited significantly higher microbial population, microbial diversity, phyto - hormone production, enzyme

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activities in station trail. Among different levels of nitrogen fertilizer applications, treatments receiving 50% organic + 50% inorganic N forms possessed significantly higher microbiota and their activities. On farm trials also exhibited similar trends as the station trial. In conclusion, the study highlights the positive effects of SRI cultivation combined with a balanced organic and inorganic nitrogen treatment on soil microbial populations and phyto-hormone production and soil enzyme activities which could have an influence of system sustainability.

Keywords: System of rice intensification (SRI); microbial population; microbial diversity siderophore; IAA; ACC deaminase; soil dehydrogenase; fluorescein diacetate hydrolase.

1. INTRODUCTION

Rice is India's most important staple food, providing approximately 60% of the daily energy requirements of the population. About 41% of the total food grain production is rice which is produced utilising 35% of the national food grain area. Improving the production and productivity of rice is crucial in ensuring national food security, as problems like reduction in cultivable land, decreasing table water, soil degradation, adverse soil and environmental conditions and climate change are currently encountered during rice production, leading to stagnation/ decline in rice crop yields. According to the present estimates, the demand for rice will be 121.2 million tonnes by 2030, 129.6 million tonnes by 2040 and 137.3 million tonnes by 2050. Consequently, to meet the rising food demands of a growing population, future rice production will need to be increased amidst limited agro-resources, especially productive land and water [1].

Rice cultivation through the 'System of Rice Intensification' (SRI) is a crop establishment method that deviates from the traditional practice of cultivating irrigated paddy [2]. The concept of SRI includes transplanting early-stage individual seedlings carefully with a spacing of 25x25 cm between each seedling under a controlled water supply regime so that the soil is well aerated, thereby promoting the growth of roots and improving biological activity in the rhizosphere. With SRI cultivation, obtaining reasonably good yields while saving 30-40 per cent of water has become possible.

The high yields and improved crop productivity recorded in SRI practice are attributed to increasing soil fertility and health due to the stimulation of beneficial microorganisms and their activities in and around rice roots. The present investigation compares the microbiological properties of rhizosphere soils of rice cultivated under SRI and conventional normal transplantation methods (NTP) under different nitrogen fertiliser management systems.

2. MATERIALS AND METHODS

Soil samples for the study were collected from a long-term (from 2008 to 2019) conducted at IIRR farm located at 17.530 N latitude, 78.270 E longitude, and an altitude of 545 meters, in ICRISAT, Patancheru, Hyderabad, India. The experimental design consisted of three replications arranged in a split-plot design. The main plots comprised two cultivation methods: SRI and NTP. The sub-plots included four nitrogen management practices: i) N_0 - Control without N application, ii) N_1 -recommended dose of nitrogen supplied in organic form, iii) N_2 - recommended dose of nitrogen supplied as a combination of organic (50%) and inorganic (50%), and iv) N_3 recommended dose of nitrogen supplied solely in inorganic form as fertiliser (Table 1). The recommended fertiliser dose for the trial was 120:60:40 kg NPK ha^{-1}. The N (urea) was applied in split doses (50% N as basal dose, 25% N at maximum tillering stage and 25% at panicle initiation stage), while the entire dose of P and K fertilisers was applied basally. The organic N source was vermicompost, applied to treatments on an N-equivalent basis. The samples were obtained from rice crop (Akshaya Dhan) grown during the *kharif* seasons of 2018 and 2019. The soil in the experimental area was black clay-loam, with a slightly alkaline pH of 7.4 (determined using a 1:2.5 soil-water suspension). It was non-saline, with an electrical conductivity (EC) of 0.715 d/Sm^{-1}, and contained 0.90% organic carbon.

Rhizosphere soil samples were also obtained from two on-farm farmers' field trials conducted at Chandepally village, Nalgonda district, Telangana, India (17.166 N latitude and 79.433 E longitude), where the rice variety DRR Dhan 44 was cultivated under SRI and NTP during the same seasons as on-farm trials (*kharif* seasons of 2018 and 2019) to record the differences in

farmers' fields. The soils in both the fields were clay-loam black with a moderately alkaline pH of 8.3. The two soils, however differed in their EC (0.145 d/Sm^{-1} and 1.525 d/Sm^{-1}) and organic carbon content (0.56% and 0.90%.).

In the farmers' field experiments, the SRI and NTP methods were considered main plots, with subplots comprising i) N1 - 100% organic and ii) N3 - 100% inorganic and iii) N2 - 50% organic + 50% inorganic fertiliser application. The recommended fertiliser dose 120:60:40 kg NPK $ha^{-1}$and the method and time of application were similar to the on-station trial.The treatments for the study conducted were as follows List 1.

2.1 Rhizosphere Soil Collection

Rice rhizospheric soil samples were collected from both station and on-farm trials during the years 2018 and 2019 during flowering stage of the crop. To represent the whole field, 4 samples were collected, composited, carried to the laboratory and preserved at 4^{o}C for further analysis [3].

2.2 Processing of Soil Samples

A tenfold serial dilution and plating technique was used to enumerate the microbial population. After serial dilution, 100 µl of aliquots of appropriate dilutions were plated on to respective media and following the viable plate count method, the colonies that appeared on the petri plates were counted and the population of different microbes were expressed as log CFU/g soil.

2.3 Enumeration of Bacteria, Fungi and Actinomycetes in Rhizosphere Soil

Nutrient Agar (NA) for bacteria, Martins Rose Bengal Agar (MRBA) for fungi and Starch Casein Agar (SCA) for actinomycetes were used for enumeration as per standard protocol [4,5,6]. After aliquots were spread on agar, the plates were incubated at 30°C and colonies that appeared on NA, MRBA and SCA after 3, 5 and 10 days were counted for bacteria, fungi and actinomycetes population log CFU gram $soil^{-1}$.

2.4 Enumeration of Phosphorous Solubilising and Siderophore Producing Bacteria

Phosphorus-solubilising microorganisms (PSMs) were enumerated using Pikovskaya's agar media. The plates were spread with a series of dilutions and were incubated at 30°C for 48-72 hours. The plates were examined for the presence of halo zone around the bacterial colonies which is an indication for solubilisation [7]. For enumeration of siderophore producing organisms, series of dilutions were spread on Chrome Azurol S (CAS) agar plates and the plates were incubated. The presence of orange halos around the bacterial colonies indicates siderophore producing organisms log CFU gram $soil^{-1}$ [8].

List 1. The treatments for the study

SRI cultivation	Conventional cultivation (NTP)
$M1N_0$- Control without fertiliser application	$M2N_0$-Control without fertiliser application
$M1N_1$- 100% organic fertiliser (N applied as vermicompost)	$M2N_1$- 100% organic fertiliser (N applied as vermicompost)
$M1N_2$- 50% Organic fertilization + 50% inorganic fertilizer	$M2N_2$– 50% Organic fertilization + 50% inorganic fertilizer
$M1N_3$ - 100% Inorganic fertilisation (N applied as urea)	$M2N_3$ – 100% Inorganic fertilisation (N applied as urea)
Farmer's Field	
M1N1 (N applied as vermicompost) 100% organic	M2N1(N applied as vermicompost)100% organic
M1N3 (N applied as urea) 100% Inorganic	M2N3 (N applied as urea) 100% Inorganic
M1N2 50% Organic fertilization + 50% inorganic fertilizer	M2N2 50% Organic fertilization + 50% inorganic fertilizer

2.5 Microbial Diversity

After different groups of microorganisms were enumerated on different media the information obtained was used to study microbial diversity using diversity indices. The Shannon-Weaver diversity index (H') denotes the distribution of species abundance within a population. H' is at its highest when all species are equally represented, and at its lowest when there's only one species in the sample. To calculate the Shannon diversity index (H') for culturable microorganisms, the equation $H = -\Sigma (Pi \times \ln Pi)$ was used, where Pi represents the proportional abundance of each type of microorganism [9]. Evenness (EH) quantifies the distribution of individuals among different species in a community, ranging from 0 to 1, with 0 indicating no evenness and 1 indicating complete evenness. To calculate species evenness, Shannon's diversity index H' is divided by the natural logarithm of species richness, ln(S).

2.6 Soil Phytohormone Production

2.6.1 Indole acetic acid content in soil

Indole acetic acid (IAA) concentrations of the soil samples were determined by using Kings B bacterial growth medium supplemented with 2.5 mM tryptophan [10]. The soil samples were inoculated in sterile broth and incubated for 7 days. IAA concentration was calculated by using the reagent which consisted of 12 g of $FeCl_3$ per litre in 7.9 M H_2SO_4. One millilitre of reagent was added to 1 ml of the sample solution, well mixed in a 3-ml spectrophotometer cuvette, and the mixture was left in the dark for 30 min at room temperature. IAA concentrations determined by absorption at 540 nm measured using a spectrophotometer.

2.7 Determination of ACC Deaminase Activity

ACC deaminase activity of was analysed by mixing 1 g soil sample (sieved to a particle size of <2mm) with 50 mL of distilled water containing 0.1 mol L^{-1} ACC (1-aminocyclopropane-1-carboxylate) and incubating the soil solution at 30°C for 24 hours [9]. After incubation, 1 mL of the solution was extracted and mixed with 1.8 mL of 0.56 mol L^{-1} hydrochloric acid followed by 0.3 mL of 0.1% (w/v) 2, 4-dinitrophenylhydrazine in 2 mol L^{-1} hydrochloric acid. After incubation at 30°C for 15 minutes, 2 mL of 2 mol L^{-1} sodium hydroxide was added to the solution and the concentration of alpha-ketobutyrate was determined by measuring the absorbance at 540nm using a spectrophotometer.

2.8 Soil Enzyme Activities

2.8.1 Dehydrogenase activity

Tris buffer (5 mL) and 1mL of 3% solution of TTC were added to 5 g soil, and after 24 hrs incubation in dark at 37°C, the triphenyl formazon (TPF) formed was extracted with 25ml of acetone [11]. Control samples were maintained without addition of TTC. The supernatants collected were used for spectrophotometric readings at 540nm against TPF (20– 100µg/ml) standards.

2.9 Fluorescein Diacetate Hydrolysis (FDA)

For FDA activity, 7.5ml of potassium phosphate buffer (pH – 7.6) and 0.1ml of substrate (FDA) was added to soil sample (1g) and incubated at 25° ºC for 30 min. Blanks are also prepared in the same manner but instead of FDA solution, 0.1ml of acetone was added and incubated. After 30 minutes, 7.5ml of chloroform and methanol (2:1 ratio) was added to both samples and blank to stop the reaction. The samples were centrifuged, supernatants were collected, and spectrometric readings were taken at 490nm against FDA standards (1- 5 µg/mL) [12].

2.10 Statistical Analysis

The experiment was set up as split plot design, the triplicate data of station trial and 5 replication data of farmers' field of the years 2018 and, 2019 was analysed statistically using pooled analysis function of OPSTAT (hau.ernet.in/about/opstat.php).

3. RESULTS AND DISCUSSION

3.1 Microbial Population in the Rice Rhizosphere

Statistically Significant higher number of bacteria were observed in SRI (9.13 log CFU gm $soil^{-1}$) in comparison to NTP (9.02 log CFU gm $soil^{-1}$) in the station trial. Among different nitrogen levels, N_3 and N_4 (50% organic fertilization + 50% inorganic fertilizer) treatment exhibited significantly higher (9.20 log CFU gm $soil^{-1}$) bacterial population (Table 1). Following the

same trend fungi and actinomycetes, were also observed to be significantly high in SRI among the planting methods, and in N_3 among the nitrogen treatments (Table 1).

Phosphorous solubilizing bacteria and siderophore producing bacteria were significantly higher in SRI (4.58 and 4.49 log CFU gm soil^{-1}repectively) method of cultivation in comparison to NTP (4.44 and 4.28 log CFU gm soil^{-1}respectively) in station trial and in 100% organic treatment (4.61 and 4.52 log CFU gm soil^{-1} respectively) which was on par with 50% organic fertilization + 50% inorganic fertilizer (4.57 and 4.45 log CFU gm soil^{-1} respectively) treatment (Table 1). When the soil from farmer's fields were analysed, similar to station trial, all the groups of microorganisms studies were significantly higher in SRI method of cultivation and in treatments with 50% organic + 50% inorganic fertiliser application (Table 2). This may be because of the increased aerobicity of the SRI soils which promote a healthy root system that invigorates microbial activities. SRI-organic + inorganic treatments where more organic fertilisers were applied compared to NTP, has been reported to enhance the population of indigenous bacteria [13,14]. Differential stimulation of soil microbial community and behaviour due to organic and conventional farming practices have also been revealed through earlier investigations [15]. When SRI planting method was followed, similar to this study, the number of beneficial microorganisms including phosphorous solubilizers and siderophore producers have been reported to increase significantly [16]. The ability of SRI rhizospheres to support higher microbial populations could be because rhizo-deposition from roots into the soil is higher in non-flooded and alternate wetting and drying regimes than in conventional continuously flooded rice systems [17].

In the station trial, endophytic bacteria were significantly higher (Table 1) in SRI roots (4.66 log CFU gm root tissue^{-1}) compared to NTP (4.50 log CFU gm root tissue^{-1}) while the roots of plants receiving 50% organic fertilization + 50% inorganic fertilizer exhibited higher endophytic bacteria (4.76 log CFU gm root tissue^{-1}) among the different nitrogen treatments (Table 2). In on farm field trials also a similar enhancement in root bacterial populations were also recorded in plants under SRI and 50% organically + 50% inorganically fertilised treatments. Significant interactive effects of establishment methods and nitrogen levels were observed for endophytic bacteria in both on-station and on-farm experiments as the highest endophytic bacterial population was supported in roots of plants grown under SRI along with application of 50% organic + 50% inorganic fertilization. Higher population of root endophytic bacteria with increased richness and diversity of endophytic bacteria was reported under non –flooded conditions compared to conventional cultivation [18].

3.2 Microbial Diversity

When the culturable microorganisms were subjected to diversity analysis it was observed that the Shannon-Weaver diversity index (H') was higher in the SRI cultivation method (1.75) than in NTP (1.74). Among the treatments with different levels of nitrogen, the 100% organic N applied treatment had a significantly higher H' index of 1.75 which was on par with the 50% organic +50% inorganic N applied treatment (1.75). The species evenness was also higher in SRI (0.98) among cultivation methods, and among the different levels of nitrogen treatment, 100% organic (0.98) N treatment was superior (Fig. 1). The diversity indices in on farm trials followed the same trends as that of the station trial (Fig 2). Microbial communities are dependent on the method of cultivation and water management. Significant increases in microbial diversity on the addition of sole organic and integrated nutrient management leading to increases in soil organic matter which in turn improves plant growth and abundant release of root exudates into the soil by plant roots [19,20] have been observed.

3.3 Soil Phytohormone (IAA and ACC Deaminase Activity) Production

Indole-3-acetic acid (IAA) is a naturally occurring plant hormone belonging to the class of auxins. When IAA is produced by soil microorganisms, it can have a positive impact on plant health. The IAA concentration (Table 3) in SRI field soils (station trial) was observed to be 2.98 µg IAA equivalents g soil^{-1}which was significantly higher than in NTP soils (2.21 µg IAA equivalents g soil^{-1}). N fertilisation also significantly influenced soil IAA content as higher concentrations of IAA was observed in soil with 50% organic + 50% inorganic N fertilisation (3.25µg IAA equivalents g soil^{-1}) followed by 100% inorganic (2.97µg IAA g soil^{-1}), 100% organic (2.31µg IAA equivalents g soil^{-1}) and control (1.84µg IAA equivalents g soil^{-1}). IAA content in soils (Table 3) from on-farms

trials were also observed to be significantly enhanced in SRI cultivation method (farmer 1:- 1.849µg IAA equivalent s g soil^{-1}, farmer 2:- 1.80µg IAA equivalents g soil^{-1}) and in treatment with 50% organic + 50% inorganic nitrogen regime (farmer 1- 1.73µg IAA equivalents g soil^{-1}, farmer 2- 1.70µg IAA equivalents g soil^{-1}).

ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) is an enzyme synthesised by specific bacteria associated with plants. It plays a crucial role in plant-microbe interactions, primarily functioning to regulate ethylene levels within plants which is stress hormone. Quantitative analysis of the soil samples from station trial revealed that ACC deaminase activity also followed the same trend as IAA as significantly higher activity was observed in SRI among planting methods and in 50% organic+ 50% inorganic treated soils (29.99 and 37.061 moL^{-1} alpha ketobutyrate g soil^{-1} day^{-1} respectively) among different N levels (Table 3). The soils obtained from farmers' fields also followed the same trend *i.e.,* SRI (farmer 1:- 14.40moL^{-1} alpha ketobutyrate g soil^{-1} day^{-1}, farmer 2:- 14.04moL^{-1} alpha ketobutyrate g soil^{-1} day^{-1}) and 50% organic + 50% inorganic (farmer 1:- 14.22moL^{-1} alpha ketobutyrate g soil^{-1} day^{-1}, farmer 2:- 12.97moL^{-1} alpha ketobutyrate g soil^{-1} day^{-1}) had highest ACC deaminase activities (Table 3). Significant interactive effects of establishment methods and nitrogen levels were observed for ACC deaminase activity in both on-station and on-farm experiments as the highest ACC deaminase activity was observed in the SRI along with 50% organic + 50% inorganic N-fertilized rhizosphere soil.

Many researchers had reported that the number of beneficial microorganisms increased under SRI cultivation which included IAA producing and ACC deaminase producing microbes [21,22,23] while a much higher stimulation is observed when SRI is combined with organic fertilisation. Additionally, differences in soil organic matter inputs have been reported to significantly impact soil IAA concentration and ACC deaminase activity [10].

3.4 Dehydrogenase (DHA) and Fluorescein Diacetate Hydrolysis Activities (FDA)

Dehydrogenase activity is an essential indicator of soil health and microbial activity. Dehydrogenases are enzymes produced by soil microorganisms, particularly bacteria and fungi, that facilitate the transfer of hydrogen atoms from organic compounds to electron acceptors, typically found in the soil. Methods of establishment and nitrogen levels were observed to have significant effect on soil dehydrogenase activity (Casida Jr. 1964).In the present study, dehydrogenase activity of the soil (station trial) was observed to be the highest in SRI, followed by NTP (2.64 and 2.05 mg TPF g^{-1} soil 24h^{-1} respectively) while dehydrogenase activity (3.93 mg TPF g^{-1} soil 24h^{-1}) in N3 was the highest among the different levels of nitrogen application(Fig. 3). Dehydrogenase activity in farmer fields also exhibited highest activity in SRI and 50% organic + 50% inorganic N fertilization treatment similar to station trial (Fig. 4).

Higher soil dehydrogenase activity due to SRI and integrated INM practices have been reported by several authors and anoxic conditions caused by flooded conditions under normal transplanted conditions has been attributed to slower enzymatic activities in NTP [24,25,26]. In addition, a strong relationship between soil organic matter content and soil enzyme activities has also been reported as soil organic carbon induces an increase in microbial processes [27,28]. Soil dehydrogenase activity could be lower under chemical fertilisation due to reduced amounts of organic substrates in these soils [29].

Fluorescein Diacetate (FDA) is a non-fluorescent compound that can penetrate living microorganisms in the soil. Once inside the microbial cells, FDA is enzymatically hydrolysed into fluorescein, a fluorescent compound. The amount of fluorescence produced is directly proportional to the activity of intracellular esterase's, providing valuable insights into the overall metabolic activity of the soil's microbial community. When the soil samples were analysed, we observed that FDA activity also followed the same trend like DHA *i.e.,* SRI and NTP methods recorded 62.60 and 60.00 µg fluorescein g^{-1} soil dry weight 0.5h^{-1}respectively. Among N1 (Control), N2 (100% organic), N3 (50% organic + 50% inorganic) and N4 levels (100% inorganic), FDA activity ranged between 45.91- 68.34 µg fluorescein g^{-1} soil dry weight 0.5h^{-1}, the lowest was in the control and highest was N3 (Figs. 5, and 6). The farmer's fields also followed the same trend as station trial. Combined use of organic and inorganic fertilisers have been reported to increase soil FDA hydrolase activity [30,31] due to enhancement in soil organic carbon content.

Table 1. Effect of planting methods and nitrogen fertilization on rice rhizospheric microbial population and root endophytic bacteria (station trial)

Treatments	Bacteria *	Fungi *	Actinomycetes *	Phosphorous solubilizing bacteria*	Siderophore producing bacteria*	Endophytic bacteria**
Methods of cultivation (M)						
SRI	9.13[a]	4.48[a]	5.40[a]	4.58[a]	4.49[a]	4.66[a]
NTP	9.02[b]	4.22[b]	5.25[b]	4.44[b]	4.28[b]	4.50[b]
CD (p=0.05)	0.03	0.25	0.16	0.08	0.07	0.05
Nitrogen fertilization (N)						
Control	8.91[c]	4.22[c]	5.26[c]	4.35[d]	4.15[c]	4.20[c]
100% organic	9.04[b]	4.29[c]	5.32[b]	4.61[a]	4.52[a]	4.68[b]
50% organic+ 50% inorganic	9.20[a]	4.51[a]	5.41[a]	4.57[b]	4.45[b]	4.78[a]
100%inorganic	9.16[a]	4.39[b]	5.36[b]	4.52[c]	4.42[b]	4.66[b]
CD (p=0.05)	0.05	0.07	0.08	0.02	0.06	0.04
Interaction						
Methods of cultivation X Nitrogen fertilization						
CD (p=0.05)	NS	NS	NS	NS	NS	0.06
Nitrogen fertilization X Methods of cultivation						
CD (p=0.05)	NS	NS	NS	NS	NS	0.07

*Where * indicates log CFU gram soil^{-1}, ** indicates log CFU gm root tissue^{-1}, variables a, b, c, d denotes significant difference and NS denotes non-significant*

Table 2. Effect of planting methods and nitrogen fertilization on rice rhizospheric microbial population and root endophytic bacteria (On-farm trial)

Treatments	Farmer 1						Farmer 2					
	Bacteria*	Fungi*	Act*	PSB*	SPB*	EB**	Bacteria*	Fungi*	Act*	PSB*	SPB*	EB**
Methods of cultivation (M)												
SRI	7.05^{a}	4.38^{a}	5.37^{a}	4.29^{a}	4.27^{a}	4.23^{a}	7.02^{a}	4.40^{a}	5.37^{a}	4.31^{a}	4.28^{a}	4.26^{a}
NTP	6.94^{b}	4.156^{b}	5.13^{b}	4.11^{b}	4.13^{b}	3.98^{b}	6.94^{b}	4.19^{b}	5.13^{b}	4.19^{b}	4.12^{b}	4.02^{b}
C.D (p=0.05)	0.02	0.04	0.03	0.05	0.01	0.06	0.01	0.03	0.03	0.03	0.05	0.03
Nitrogen fertilization (N)												
100% organic	6.96^{c}	4.17^{c}	5.15^{c}	4.12^{c}	4.11^{c}	4.00^{c}	6.94^{c}	4.21^{c}	5.15^{c}	4.17^{b}	4.19^{b}	4.06^{c}
100% inorganic	6.99^{b}	4.29^{b}	5.29^{b}	4.21^{b}	4.21^{b}	4.10^{b}	6.98^{b}	4.30^{b}	5.27^{b}	4.27^{a}	4.16^{b}	4.14^{b}
50% organic + 50% inorganic	7.03^{a}	4.35^{a}	5.32^{a}	4.28^{a}	4.28^{a}	4.22^{a}	7.02^{a}	4.36^{a}	5.34^{a}	4.31^{a}	4.24^{a}	4.22^{a}
C.D (p=0.05)	0.02	0.04	0.03	0.04	0.05	0.03	0.01	0.03	0.05	0.05	0.04	0.04
Interaction												
Methods of cultivation X Nitrogen fertilization												
C.D (p=0.05)	NS	NS	NS	NS	0.31	0.05	NS	NS	NS	NS	0.31	0.05
Nitrogen fertilization X Methods of cultivation												
C.D (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Where Act denotes actinomycetes, PSB denotes phosphorous solubilizing bacteria, SPB denotes siderophore producing bacteria, EB denotes endophytic bacteria, * indicates log CFU gram soil^{-1}, ** indicates log CFU gm root tissue^{-1}, and variables a, b, c, d denotes significant difference and NS denotes non-significant*

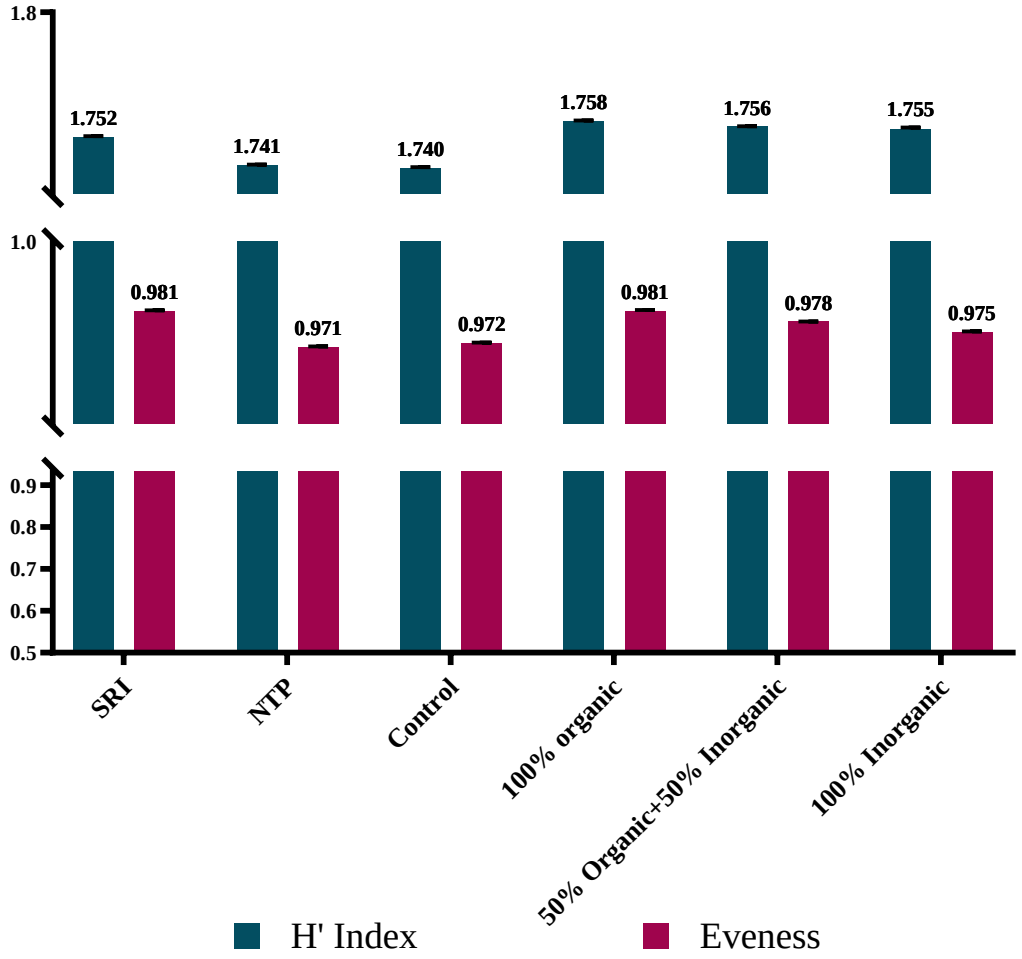

Fig. 1. Effect of planting methods and nitrogen fertilization on microbial diversity (station trial)

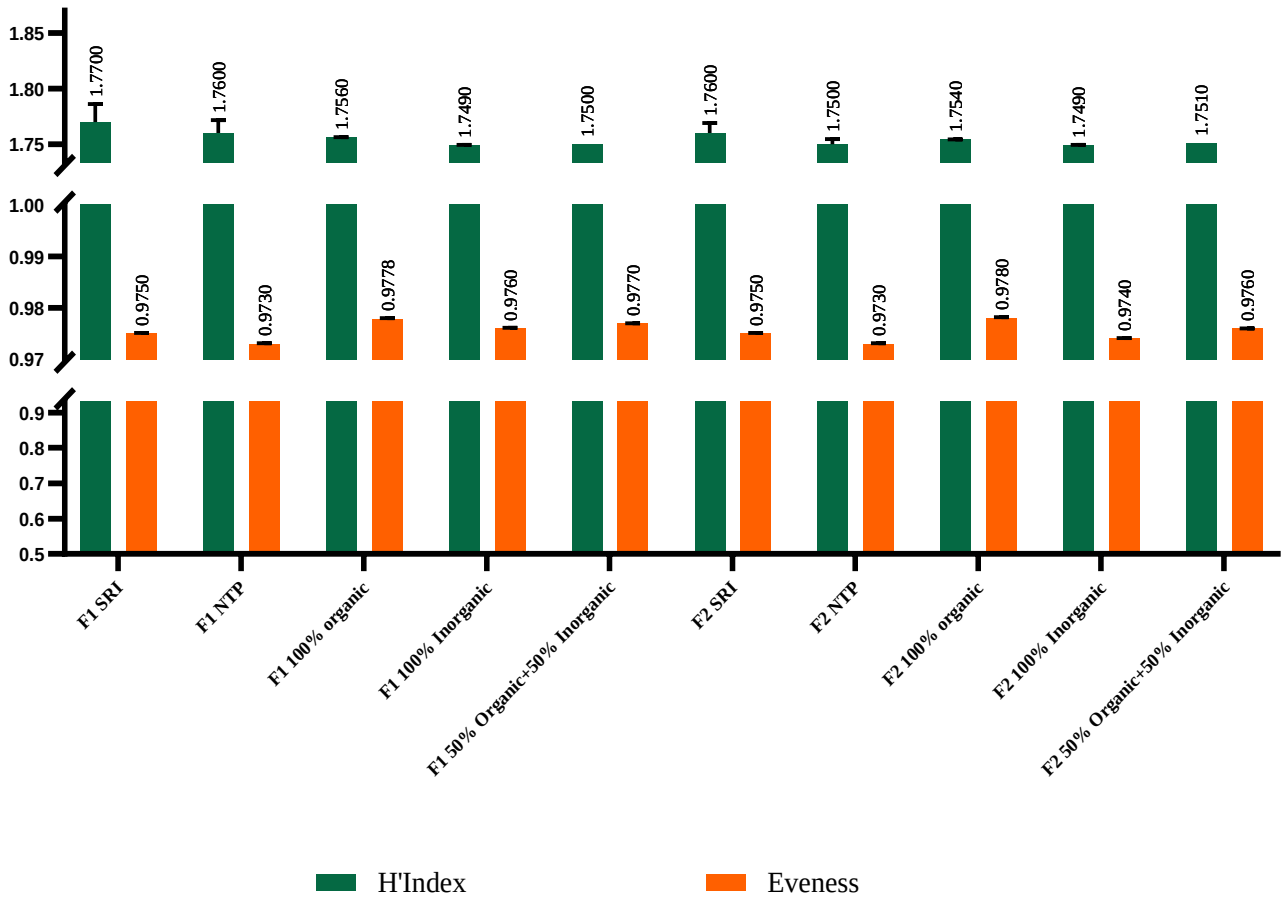

Fig. 2. Effect of planting methods and nitrogen fertilization on Microbial diversity (On-farm trial) (F1: Farmer 1 F2: Farmer 2)

Table 3. Effect of planting methods and nitrogen fertilization on IAA and ACC content in rice rhizosphere (station and on-farm trials)

Treatments	IAA concentration**			ACC deaminase activity***		
	Station trial	Farmer 1	Farmer 2	Station trial	Farmer 1	Farmer 2
Methods of cultivation (M)						
SRI	2.98[a]	1.85[a]	1.80[a]	29.99[a]	14.40[a]	14.04[a]
NTP	2.21[b]	1.50[b]	1.47[b]	28.67[b]	12.60[b]	11.45[b]
C.D (p=0.05)	0.18	0.24	0.18	0.20	0.22	0.94
Nitrogen fertilization (N)						
Control	1.84[c]	NA	NA	21.60[d]	NA	NA
100% organic	2.31[b]	1.61[a]	1.59[a]	26.25[c]	12.79[c]	12.55[a]
50% organic+ 50% inorganic	3.25[a]	1.73[a]	1.70[a]	37.07[a]	14.22[a]	12.97[a]
100%inorganic	2.97[a]	1.68[a]	1.62[a]	32.40[b]	13.50[b]	12.72[a]
C.D (p=0.05)	0.34	0.00	0.00	1.41	0.31	0.00
Interaction						
Methods of cultivation X Nitrogen fertilization						
C.D (p=0.05)	NS	NS	NS	0.23	0.25	0.83
Nitrogen fertilization X Methods of cultivation						
C.D (p=0.05)	NS	NS	NS	NS	0.27	0.91

*** indicates µg IAA equivalents gram soil^{-1}, *** indicates moL^{-1} alpha ketobutyrate gram soil^{-1} day^{-1}and variables a, b, c, d denotes significant difference and NA denotes the treatment is not taken up in the described field, NS denotes non-significant*

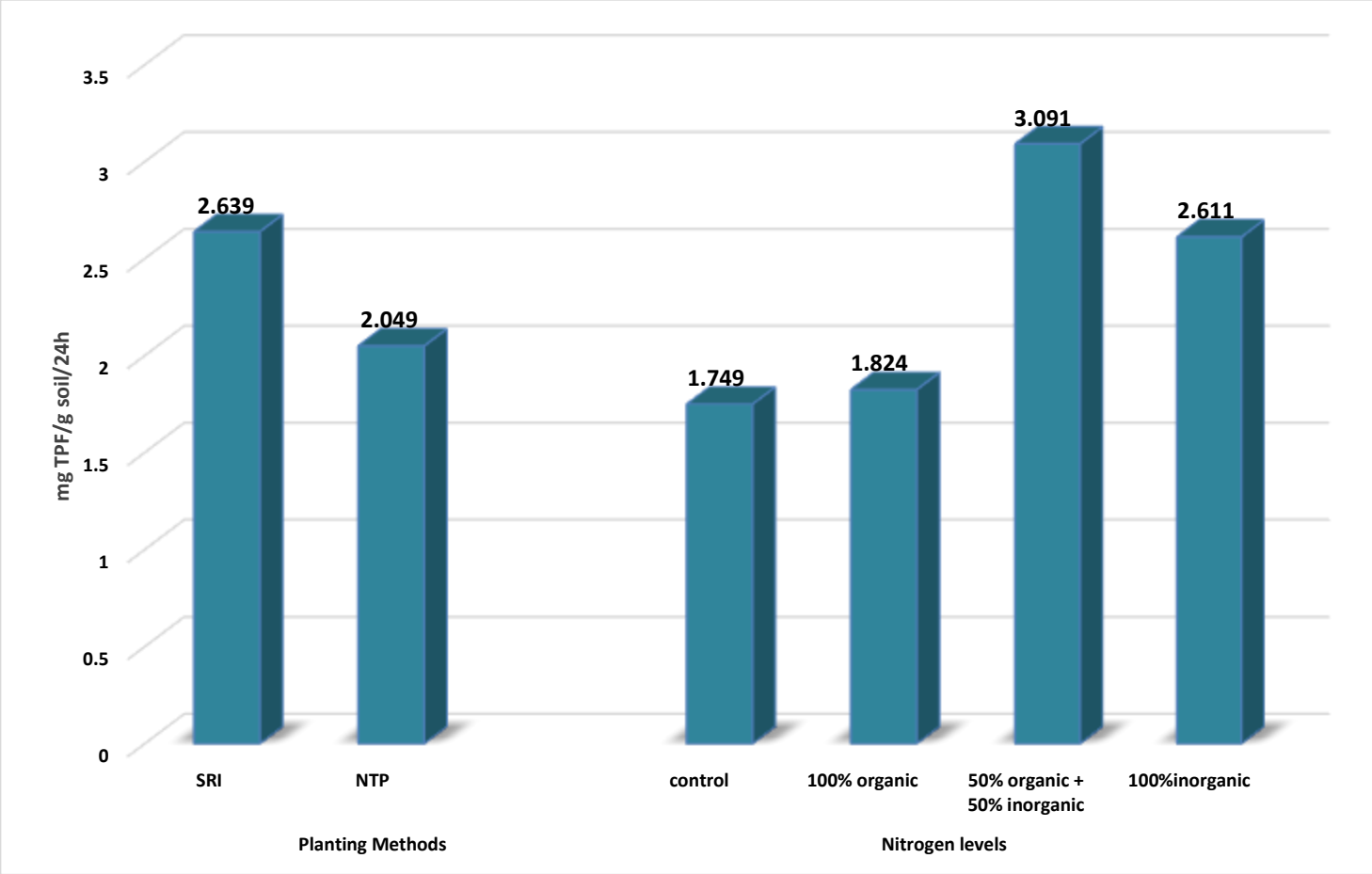

Fig. 3. Effect of planting methods and nitrogen fertilization on soil dehydrogenase activity (station trial)

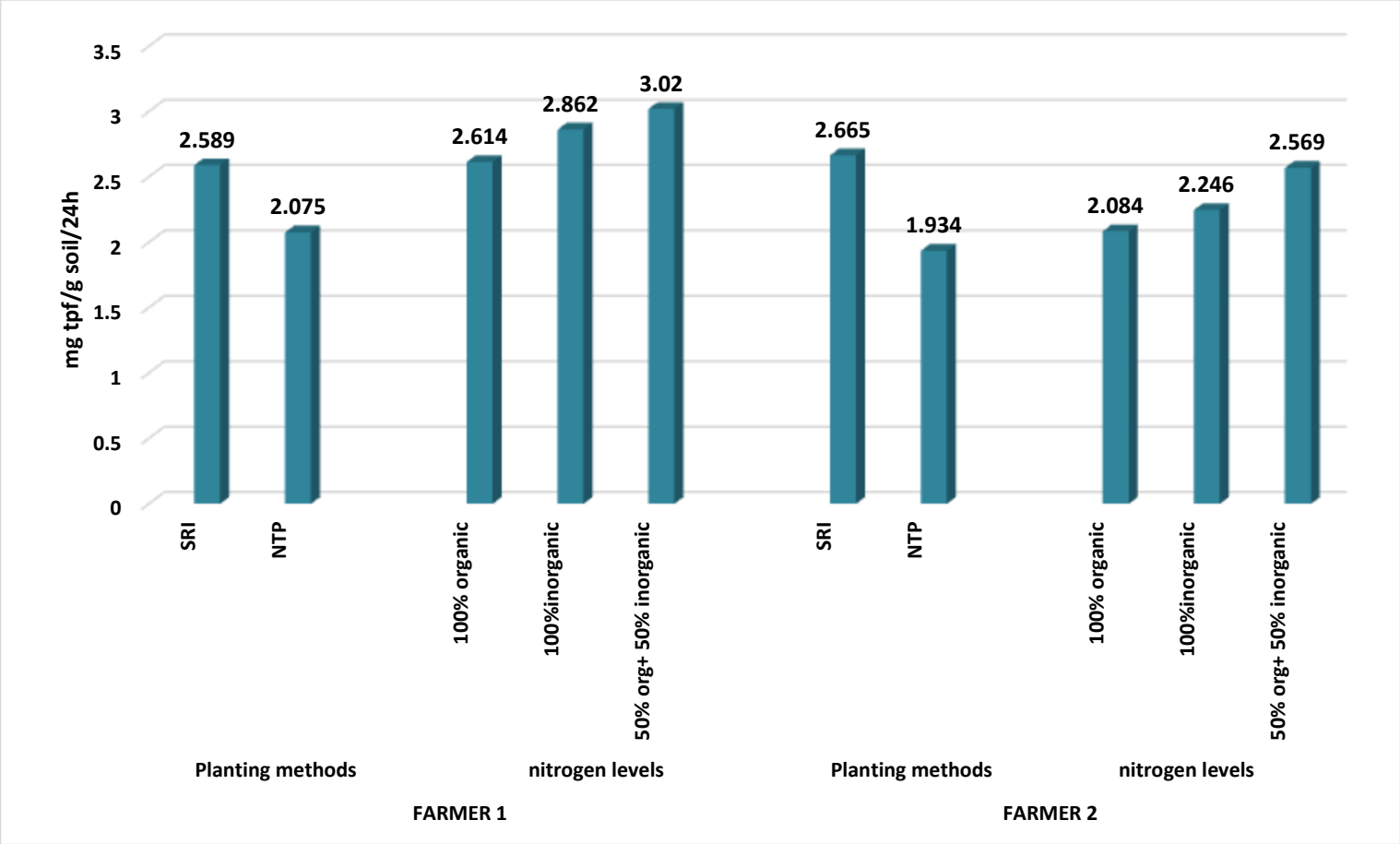

Fig. 4. Effect of planting methods and nitrogen fertilization on soil dehydrogenase activity (on-farm trial)

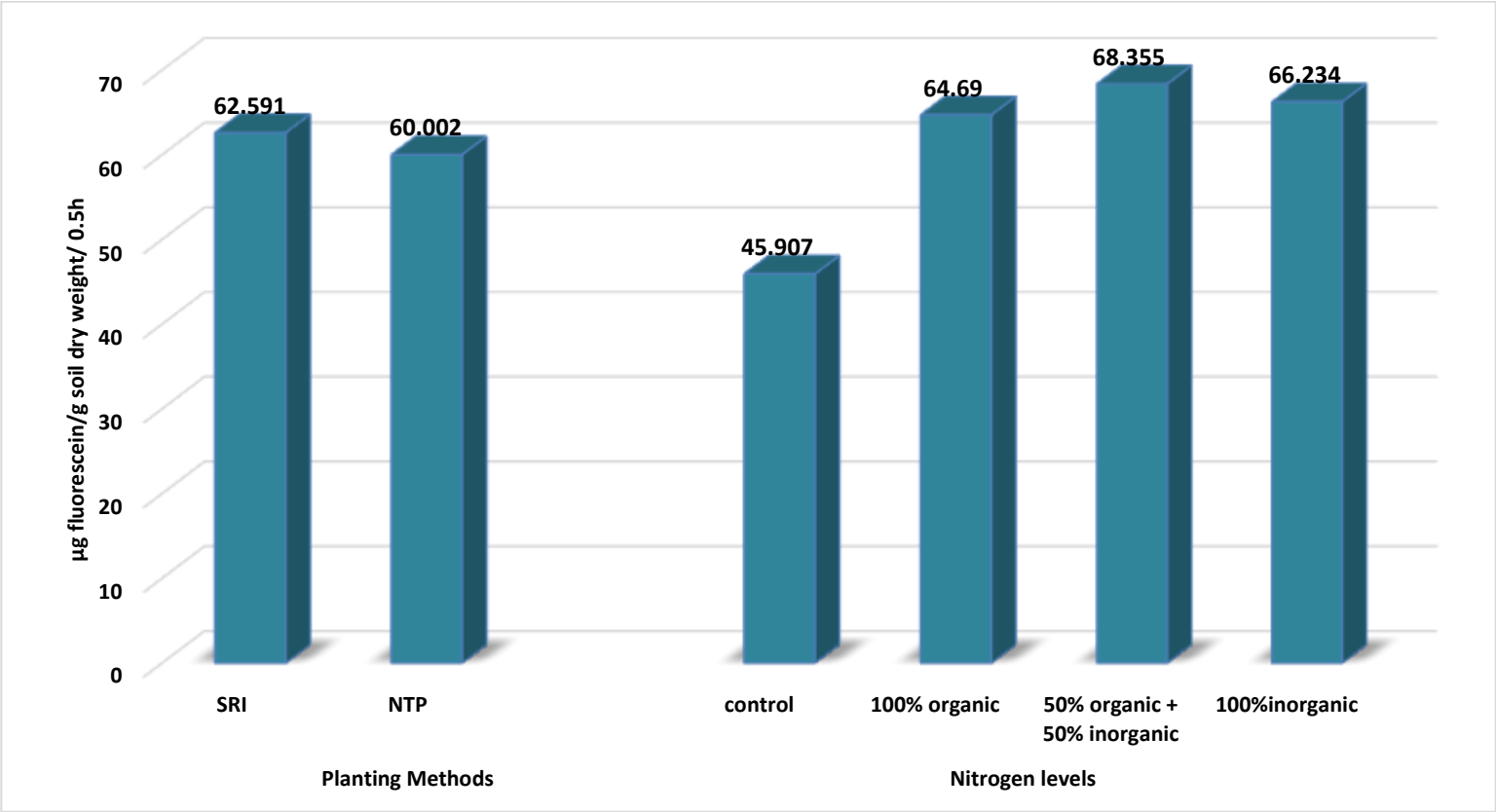

Fig. 5. Effect of planting methods and nitrogen fertilization on Fluorescein Diacetate Hydrolysis (station trial)

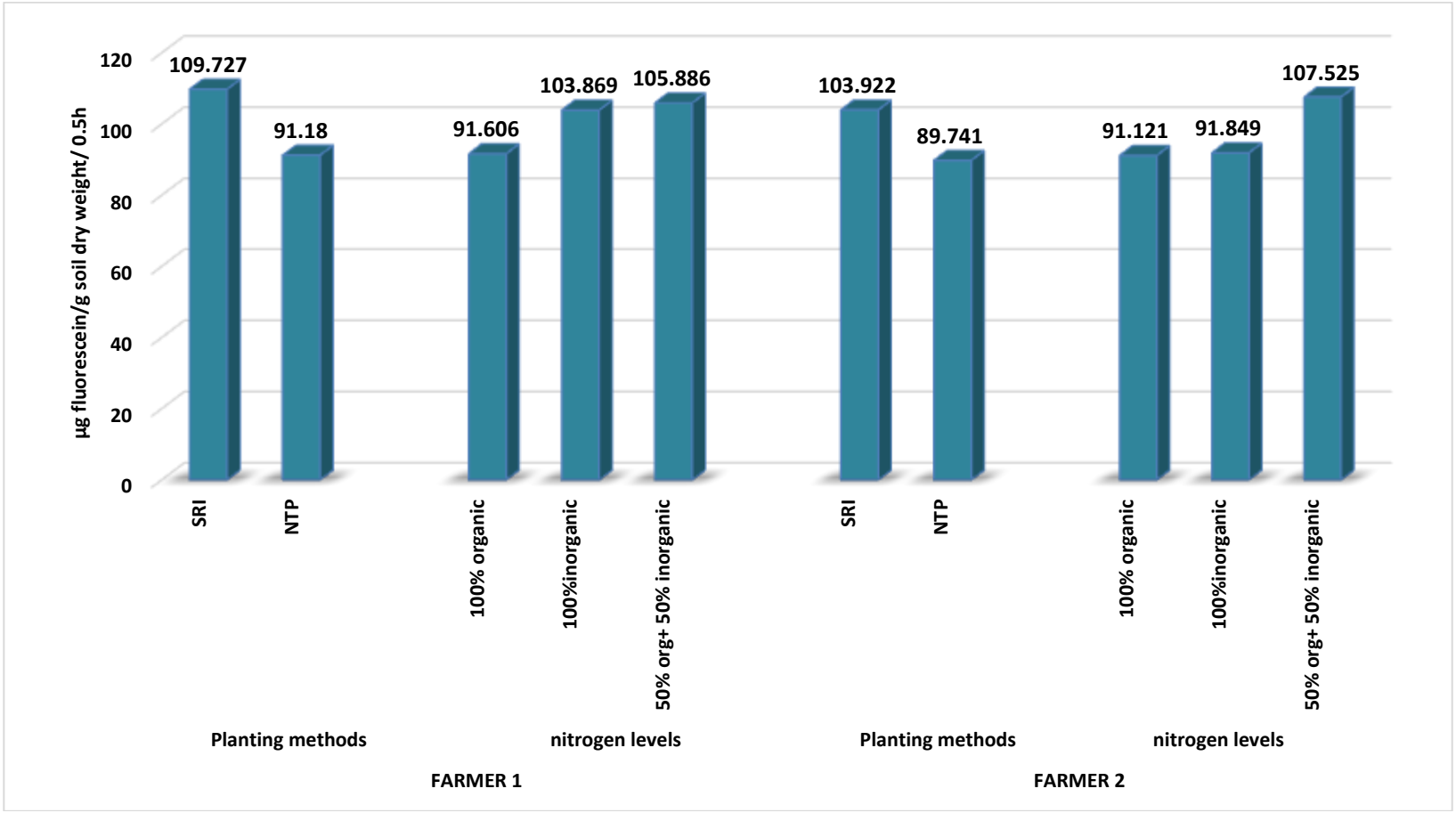

Fig. 6. Effect of planting methods and nitrogen fertilization on Fluorescein Diacetate Hydrolysis (on-farm trial)

4. CONCLUSION

In conclusion, this study demonstrates the significant impact of System of Rice Intensification (SRI) cultivation and combined use of 50% organic and 50% inorganic nitrogen treatment on soil microbial population, diversity and activities in both station and on farm trials. Overall, findings highlight the positive impact of SRI cultivation on soil health and underline the importance of a balanced organic and inorganic nitrogen treatment for maximising the benefits of SRI. Implementing SRI with appropriate nitrogen management has the potential to enhance soil microbial populations, soil phytohormone and enzyme activities, ultimately contributing to sustainable and more productive agricultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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