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Effect of combined use of organic and inorganic sources of nutrients on soil biological indicators and yield in aerobic rice (*Oryza sativa*)

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ABSTRACT

A field experiment was conducted during the summer 2022 and *navarai* (December 2022–April 2023) seasons at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu to evaluate the impact of different sources of nutrients on soil biological indicators and yield of rice (*Oryza sativa* L.) under aerobic conditions. Cultivation of aerobic rice with and without *daincha* (*Sesbania aculeate*) intercropping along with different sources of nutrients [M_1, Rice alone and M_2, Rice + *Dhaincha* intercropping and sub plot treatments were S_1, Control; S_2, Full dose of RDN (Recommended Dose of Nitrogen) from inorganic fertilizer; S_3, Half dose of RDN from inorganic fertilizer + half dose of RDN from enriched farmyard manure (EFYM); S_4, Half dose of RDN from inorganic fertilizer + half dose of RDN from EFYM + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ at 25 and 45 DAS); S_5, Half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from vermicompost (VC), top-dressed at 25 DAS and S_6, Half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from VC + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ at 25 and 45 DAS)] were tested in a split plot design (SPD) replicated thrice. In both the seasons, incorporation of *daincha* (M_2) along with half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from VC + AM fungi + foliar nutrients of 0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ (S_6) recorded the highest soil microbial biomass, enzymes activity and yield (3777 and 4599 kg/ha, respectively) of rice under aerobic condition. Therefore, combined use of organic and inorganic nutrient sources serves as a sustainable and effective strategy to improve soil health, enhance biological indicators and increase the yield of aerobic rice.

Keywords: Aerobic rice, AM fungi, Organic sources, Sustainability

In paddy (*Oryza sativa* L.), cultivation optimising the usage of organic manures and fertilizers for maximum nutrient recovery is one of the challenging ways for enhancing the productivity of rice. While supplying additional nutrients, priority should be given mainly to the organic sources of nutrients such as vermicompost, enriched farmyard manure (FYM) and green manure in addition with inorganic fertilizers, which paves the way for long-term soil fertility and productivity. Further, it would be a better option for on-farm resource recycling and utilisation repeatedly and also improving physical properties of soil (Dass *et al.* 2009, Basha *et al.* 2017).

Soil inherent ability on nutrient suppling capacity directly supports the plant physiological and morphological development. Optimal crop growth and yield is determined not only on the soil structural and chemical properties but also on its microbial activity. Soil microbial population, plays a vital role in agriculture, which ensures the nutrient availability to crop. The presence of soil microbes mediates the complex organic matter, undergoes certain biochemical changes in soil such as decomposition of organic compounds, mineralization of nutrients, and solubilization of fixed minerals. These always support the key ecosystem activities and stabilise the soil structures. Soil enzymes are constantly working for the fertility, restoration, physical and chemical stability and other ecological conditions of the soil (Patra *et al.* 2020). These enzymes are essential for many biochemical processes that are involved in the general breakdown of organic complex in soil (Kumar *et al.* 2021). Study on soil biological quality is important one when researching on different levels of nutrient application with different sources. By focusing on improving the soil biological parameters, combined application of organic and inorganic sources of nutrients greatly helps to increase the soil enzymatic activity by microflora under rice cultivation. Aerobic rice systems are being increasingly adopted to conserve water and reduce methane emissions compared to conventional flooded rice systems. However, the sustainability of this system is constrained by a gradual decline in soil fertility and biological activity, which lead to reduced productivity

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over time. Although several nutrient management strategies, including the application of organic and inorganic sources, have been tested, there is limited understanding of the synergistic effects of their combined use on soil biological indicators and yield. This study aims to address these gaps by evaluating the effects of integrated nutrient management, including microbial biomass and enzyme activity, on soil health and productivity in aerobic rice cultivation.

MATERIALS AND METHODS

A field experiment was conducted during the summer 2022 and *navarai* (December 2022–April 2023) seasons at Tamil Nadu Agricultural University, Coimbatore (11°N, 77° E), Tamil Nadu. The soil of the experimental site had a clay loam texture and was moderately alkaline, with *p*H values of 8.1 and 8.0 and EC of 0.49 and 0.47 dS/m. Initial soil fertility analysis indicated low levels of available $KMnO_4$-N (210 and 222 kg/ha), medium levels of available Olsen-P (20.2 and 20.5 kg/ha) and high levels of available NH_4OAc-K (690 and 679 kg/ha) during the summer (2022) and *navarai* (2023) seasons, respectively. The field experiment followed a split-plot design (SPD) with 12 treatments, replicated three times and individual plot sizes of 6 m × 3 m. Main plot treatments included M_1, Rice alone and M_2, Rice + *Dhaincha* intercropping and sub plot treatments were S_1, Control; S_2, Full dose of RDN (Recommended Dose of Nitrogen) from inorganic fertilizer; S_3, Half dose of RDN from inorganic fertilizer + half dose of RDN from enriched farmyard manure (EFYM); S_4, Half dose of RDN from inorganic fertilizer + half dose of RDN from EFYM + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ at 25 and 45 DAS); S_5, Half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from vermicompost (VC), top-dressed at 25 DAS and S_6, Half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from VC + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ at 25 and 45 DAS). Seeds of the rice variety CO 53 were directly sown in dry soil at a spacing of 20 cm × 10 cm. *Dhaincha* seeds were sown simultaneously in an additive series at a 1:1 ratio, following the treatment schedule. The *dhaincha* plants were manually incorporated into the soil at 25 DAS. Organic amendments such as enriched farmyard manure (EFYM), vermicompost (VC) and AM fungi were applied before sowing as per treatments. The irrigation was given to the crop immediately after sowing and the next irrigation was provided on 3 DAS. To maintain optimal soil moisture levels, the plots were kept saturated without impounding water. This was achieved by providing a 5 cm depth of irrigation twice in every week. The TNAU recommended dose of fertilizers (150:50:50 kg/ha) was given on split basis. In aerobic rice cultivation, pendimethalin at 1.0 kg/ha was applied, followed by bispyribac sodium at 20 g/ha, along with one hand weeding. Pest and disease management was done as per recommended package of practices of the region.

Analysis of soil biological indicators: The rhizosphere soil was collected at different growth stages of crop for estimation of soil microbial population count and soil enzymatic activity. Total bacterial population count was estimated by using serial dilution and plating method with nutrient agar medium and expressed in cfu × 10^{-6}/g of soil (Collings and Lyne 1968), total fungi population using Martin's Rose Bengal agar medium (Martin 1950) and total actinomycetes using Kenknight's agar medium and expressed as cfu/g of soil (Kenknight and Muncie 1939). Soil urease activity was measured using a spectrophotometer at 630 nm following the method of Tabatabai and Bremner (1972). Dehydrogenase activity was assessed at 485 nm based on the procedure by Casida *et al.* (1964). Acid and alkaline phosphatase activities were determined at 420 nm following the method of Tabatabai and Bremner (1969).

Statistical analysis: Data were analysed by using SPSS 16.0. software. As there existed a wide variation in original data of microbial population, data were transformed before the ANOVA by square root transformation $\sqrt{x + 0.5}$. Among the treatments, wherever, statistical difference was observed, the critical difference (CD) was calculated for comparing at five percent probability level and the p values were recorded. Pearson correlation analysis was performed using R software (version 4.2.0; R Studio 2022.02.3+492) to assess the relationship between microbial populations and soil enzyme activities in the aerobic rice rhizosphere.

RESULTS AND DISCUSSION

Yield of aerobic rice: Intercropping of *dhaincha* (M_2) had a significant impact on grain yield (GY) (2552 and 2432 kg/ha), straw yield (SY) (3435 and 3355 kg/ha) and harvest index (HI) (0.42 and 0.42) of rice during both summer and *navarai* seasons, respectively.

During summer season, among the different INM practices, half dose (RDN) from inorganic fertilizer + quarter dose (RDN) from EFYM + quarter dose (RDN) from VC + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$) (S_6) recorded significantly higher GY (3777 kg/ha), SY (4599 kg/ha) and HI (0.45) than other nutrient treatment combinations. This was followed by S_4. The lowest GY (1423 kg/ha) and SY (2391 kg/ha) and HI (0.37) were recorded in control (S_1). Similar trend was recorded during *navarai* season also.

Interaction effect between intercropping of *dhaincha* and INM practices on grain and straw yield of aerobic rice was significant during summer and *navarai* seasons. The combined application of organic manures and inorganic fertilizers provided a balanced nutrient supply, which contributed to higher productivity in aerobic rice. In the current study, the results revealed that *dhaincha* intercropping along with application of half dose (RDN) from inorganic fertilizer + quarter dose (RDN) from EFYM + quarter dose (RDN) from VC + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$) (M_2S_6) exhibited significantly higher values for grain and straw yields and HI (Table 1). The significant yield improvement could be attributed to the balanced nutrient supply provided by the combined use of

Table 1 Effect of combined application of organic and inorganic sources of nutrients on yield of aerobic rice

Treatments	Summer (2022) (kg/ha)			*Navarai* (2023) (kg/ha)		
	GY	SY	HI*	GY	SY	HI*
M_1	2296	3209	0.41	2240	3091	0.40
M_2	2552	3435	0.42	2432	3355	0.42
SEd	40	50	-	35	59	-
CD (p=0.05)	173	216	-	151	254	-
S_1	1423	2391	0.37	1403	2351	0.36
S_2	1878	3057	0.38	1808	2917	0.38
S_3	1758	2944	0.37	1738	2849	0.37
S_4	2854	3495	0.45	2734	3416	0.45
S_5	2857	3448	0.45	2687	3347	0.44
S_6	3777	4599	0.45	3647	4459	0.45
SEd	56	86	-	56	84	-
CD (p=0.05)	117	181	-	118	176	-
M at S						
SEd	79	122	-	80	119	-
CD (p=0.05)	165	256	-	166	249	-

Treatment details are given under Materials and Methods.
GY, Grain yield; SY, Straw yield; HI, Harvesr index.

organic and inorganic nutrient sources (Midya *et al.* 2021). The potential increase in grain yield could be ascribed to the enhancement in plant height, leaf area index, dry matter production and photosynthetic rate which might have contributed for improved allocation of carbohydrates from the leaves to the reproductive structures (Ram *et al.* 2020). The presence of mycorrhizal fungi significantly increased the grain and straw yield by establishing a symbiotic relationship with the plant roots, there by mycorrhizal fungi might have facilitated the uptake of nutrients, particularly phosphorus from the soil. This improved nutrient acquisition lead to enhanced growth and yield attributes which in turn lead to higher grain and straw yield of rice plants (Aziez 2022).

Soil microbial populations: During summer season, significantly higher bacterial (6.44, 7.06, 7.49 and 6.74 × 10^6 CFU/g), fungal (3.70, 4.38, 4.93 and 3.99 ×10^3 CFU/g) and actinomycetes population (5.35, 6.49, 6.53 and 6.12 × 10^4 CFU/g) were recorded in *dhaincha* intercropping (M_2) at 30, 60, 90 DAS ant at harvest stage, respectively than sole rice (M_1). The results were similar during *navarai* season.

At 30 DAS during both summer and *navarai* season, INM practices, viz. inorganic fertilizer, EFYM, VC, AM fungi and foliar application of nutrients (S_6) recorded significantly higher bacterial (6.89 and 6.97 × 10^6 CFU/g, respectively), fungal (4.07 and 4.05 × 10^3 CFU/g, respectively) and actinomycetes population (6.07 and 5.95 × 10^4 CFU/g, respectively) than other treatments. The least soil microbial population were in control plot (S_1). At 60, 90 DAS and harvest stage of rice also, same trend of results were recorded (Table 2 and 3).

With respect to interaction effect between *dhaincha* intercropping and nutrient management practices on soil microbial populations, there was no significant interaction at 30, 60, 90 DAS and at harvest stage in both the seasons.

Dhaincha intercropping (M_2) had a positive impact on soil biological indicators. The high organic carbon content in soil treated with *dhaincha* enhanced microbial activity by supplying carbon, energy and essential nutrients necessary for their growth and reproduction (Munda *et al.* 2018, Ajaykumar and Thavaprakaash 2020).

The combined application of half dose (RDN) from inorganic fertilizer + quarter dose (RDN) from EFYM +

Table 2 Effect of treatments on changes in soil microbial population (Summer 2022)

Treatment	Bacteria (10^6 CFU/g)				Fungi (10^3 CFU/g)				Actinomycetes (10^4 CFU/g)			
	30 DAS	60 DAS	90 DAS	Harvest	30 DAS	60 DAS	90 DAS	Harvest	30 DAS	60 DAS	90 DAS	Harvest
M_1	5.86	6.54	6.97	6.44	3.06	3.74	4.01	3.68	5.02	5.77	6.04	5.63
M_2	6.44	7.06	7.49	6.74	3.70	4.38	4.93	3.99	5.35	6.49	6.53	6.12
SEd	0.09	0.11	0.25	0.07	0.07	0.44	0.07	0.06	0.08	0.14	0.10	0.09
CD (p=0.05)	0.41	0.49	1.06	0.32	0.29	1.88	0.32	0.27	0.32	0.58	0.45	0.39
S_1	5.47	5.95	6.35	5.64	2.75	3.39	3.79	3.03	4.36	5.18	5.20	4.69
S_2	5.62	6.20	6.87	6.06	3.00	3.67	4.00	3.24	4.64	5.45	5.66	5.31
S_3	6.01	6.76	7.23	6.50	3.25	3.95	4.42	3.75	5.04	6.00	6.11	5.80
S_4	6.28	6.98	7.43	6.85	3.53	4.27	4.65	4.05	5.35	6.44	6.50	5.99
S_5	6.62	7.21	7.53	7.13	3.68	4.41	4.86	4.32	5.64	6.70	6.96	6.62
S_6	6.89	7.70	7.95	7.37	4.07	4.65	5.10	4.63	6.07	7.00	7.27	6.82
SEd	0.20	0.25	0.29	0.23	0.09	0.60	0.18	0.14	0.17	0.19	0.27	0.30
CD (p=0.05)	0.42	0.53	0.60	0.48	0.19	1.25	0.38	0.29	0.36	0.39	0.56	0.63
M at S												
SEd	0.28	0.36	0.41	0.33	0.13	0.85	0.26	0.20	0.24	0.26	0.38	0.43
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment details are given under Materials and Methods.

Table 3 Effect of treatments on changes in soil microbial population (*Navarai* 2023)

Treatments	Bacteria (10^6 CFU/g)				Fungi (10^3 CFU/g)				Actinomycetes (10^4 CFU/g)			
	30 DAS	60 DAS	90 DAS	Harvest	30 DAS	60 DAS	90 DAS	Harvest	30 DAS	60 DAS	90 DAS	Harvest
M_1	5.89	6.27	6.44	5.96	3.21	3.62	3.88	3.47	5.00	5.15	5.47	5.11
M_2	6.28	6.74	6.98	6.55	3.57	3.93	4.20	3.76	5.35	5.61	5.88	5.52
SEd	0.09	0.11	0.11	0.10	0.06	0.06	0.07	0.06	0.07	0.09	0.09	0.09
CD (p=0.05)	0.38	0.47	0.48	0.45	0.24	0.27	0.29	0.26	0.30	0.39	0.41	0.39
S_1	4.95	5.64	5.93	5.18	2.59	3.16	3.47	2.69	4.43	4.51	4.66	4.14
S_2	5.65	5.91	6.17	5.84	2.94	3.49	3.77	3.16	4.74	4.95	5.22	4.89
S_3	6.02	6.52	6.51	6.18	3.27	3.67	3.97	3.54	5.05	5.25	5.60	5.28
S_4	6.28	6.96	6.83	6.43	3.65	4.10	4.21	4.12	5.28	5.57	5.83	5.55
S_5	6.65	6.77	7.16	6.72	3.82	3.90	4.26	3.92	5.63	5.88	6.17	5.89
S_6	6.97	7.24	7.66	7.16	4.05	4.33	4.56	4.29	5.95	6.21	6.54	6.14
SEd	0.2	0.23	0.28	0.28	0.11	0.12	0.14	0.16	0.23	0.26	0.26	0.17
CD (p=0.05)	0.42	0.49	0.59	0.59	0.24	0.24	0.29	0.32	0.49	0.54	0.55	0.35
M at S												
SEd	0.28	0.34	0.40	0.40	0.17	0.17	0.19	0.22	0.33	0.37	0.37	0.24
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Treatment details are given under Materials and Methods.

quarter dose (RDN) from VC + AM fungi + foliar nutrients (0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$) (S_6) recorded maximum soil microbial population. Whereas minimum soil microbial population was observed in 100% RDN through inorganic fertilizer. The soil microbial populations were found to increase as a result of applying organic manures and green manure. This increase in microbial activity could be attributed to the higher organic carbon content in the soil resulting from the application of organic manures, in comparison to the use of inorganic fertilizers alone. The decreased abundance of soil microbial communities observed in recommended dose of fertilizers could be attributed to the inhibitory properties of chemical fertilizers, which hinder the growth and development of microorganisms (Singh and Hamimed 2022). AM fungi play a crucial role in enhancing soil fertility by enhancing the interactions with various microorganisms within the soil. Secretions produced by AM fungi have a significant impact on the composition and functioning of microbial communities present in the rhizosphere (Fall *et al.* 2022).

Soil enzymes activity: At 30 DAS during both summer and *navarai* season, significantly higher urease (21.3 and 16.4 µg NH_4^+ - N/g soil/h, respectively), dehydrogenase (22.7 and 22.3 µg/24 h/ g, respectively), acid phosphatase (57.1 and 54.44 µg p-nitrophenol/g/h, respectively) and alkaline phosphatase activity (66.9 and 67.4 µg p-nitrophenol/g/h, respectively) were recorded with *dhaincha* intercropping (M_2) compared to sole rice (M_1). Similar trend of results were observed at 60, 90 DAS and harvest stage in both the seasons (Fig. 1).

Among the treatment combinations, S_6 (inorganic fertilizer, EFYM, VC, AM fungi and foliar application of nutrients) recorded significantly higher enzymatic activity over the other treatments. During summer and *navarai* seasons, the maximum soil enzymatic activity such as urease (26.5 and 21.5 µg NH_4^+ - N/g soil/h, respectively), dehydrogenase (29.1 and 28.2 µg/24 h/g, respectively), acid phosphatase (61.2 and 61.54 µg p-nitrophenol/g/h, respectively) and alkaline phosphatase (80.4 and 75.4 µg p-nitrophenol/g/h, respectively) were observed at 30 DAS. The lowest enzymes activity was observed in control (S_1). Same trend of results was observed at 60, 90 DAS and harvest stage in both the seasons. Interaction effect between *dhaincha* intercropping and INM practices on soil enzymes activity was non-significant at 30, 60, 90 DAS and at harvest stage in both the seasons.

The soil microorganisms under *dhaincha* intercropping were stimulated by the presence of a substantial amount of organic carbon. This organic carbon serves as a source of carbon, energy and essential nutrients necessary for microbial growth and reproduction. This, in turn, stimulates microbial activity, leading to an increase in soil enzyme activity (Nandy *et al.* 2023). Soil enzymes activity significantly increased with the application of inorganic fertilizer, EFYM, VC, AM fungi and foliar application of nutrients (S_6). This might be due to the fact that organic manure serves as the primary and exclusive supplier of carbon and energy for heterotrophic organisms. It effectively furnishes abundant nutrients that support the proliferation of microbes and their metabolic activities, particularly in relation to soil enzymes. The lowest enzymes activity was observed in the control plot. This might be due to the lack of adequate substrate, specifically organic carbon. This organic carbon serves as a vital food source for the proliferation and growth of microbial populations (Kumari *et al.* 2017).

Integrated nutrient management along with AM fungi

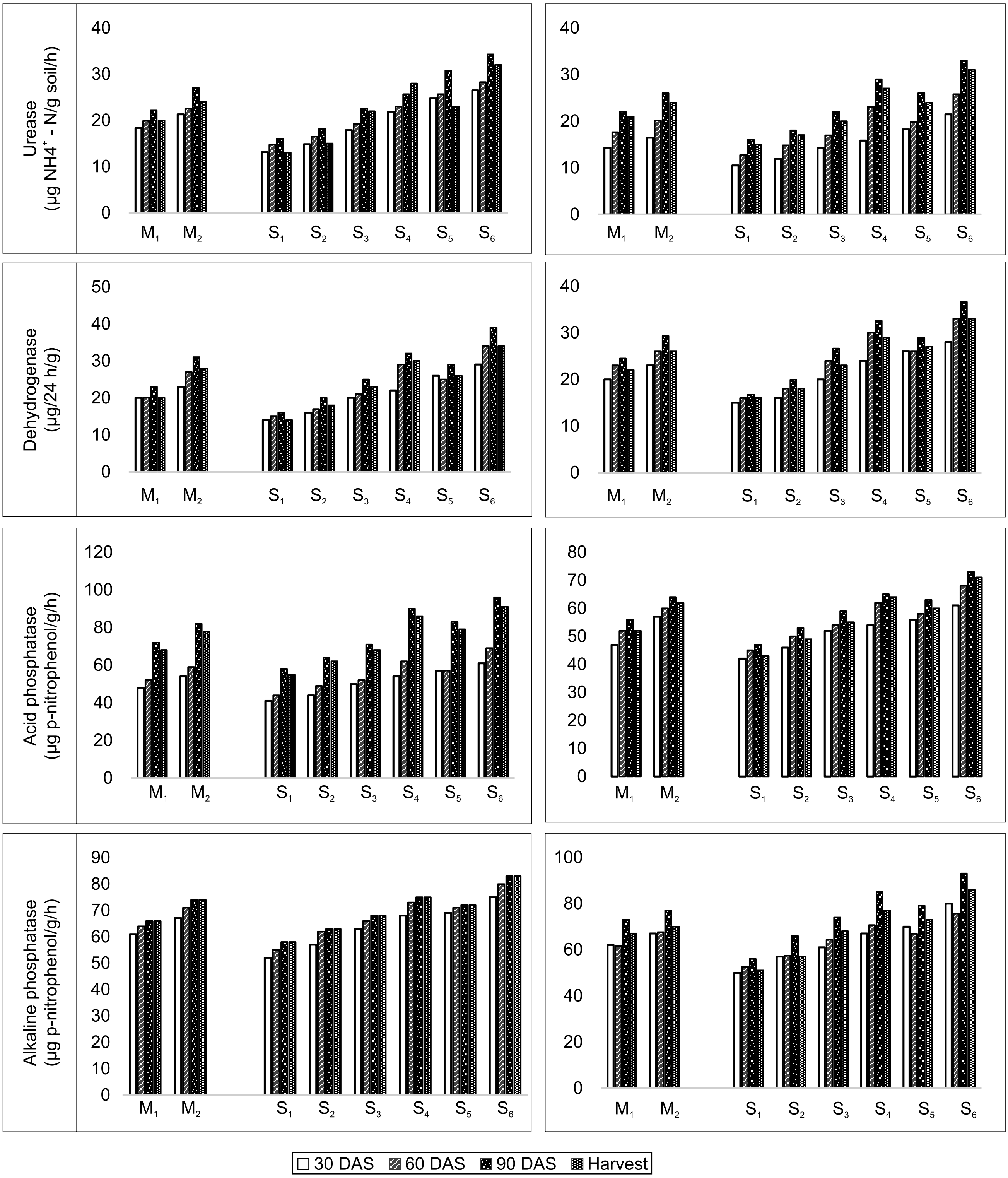

Fig. 1 Effect on changes in soil enzymes (Summer 2022 and *Navarai* 2023).
Treatment details are given under Materials and Methods.

application improves soil enzymes activity in soil. This might be due effects on soil enzyme activity, may not solely be attributed to the physical presence of hyphae, but also to the chemical compounds released by the fungi and plant roots (Darjee *et al.* 2023). Integrated nutrient management treatments exhibited higher dehydrogenase enzyme activity due to degradation of organic material and released a variety of compounds that serve as substrates for microbial growth and metabolism. As a result, the increased availability of these substrates stimulated the production and activity of microbial enzymes, including dehydrogenases (Iqbal *et al.* 2022).

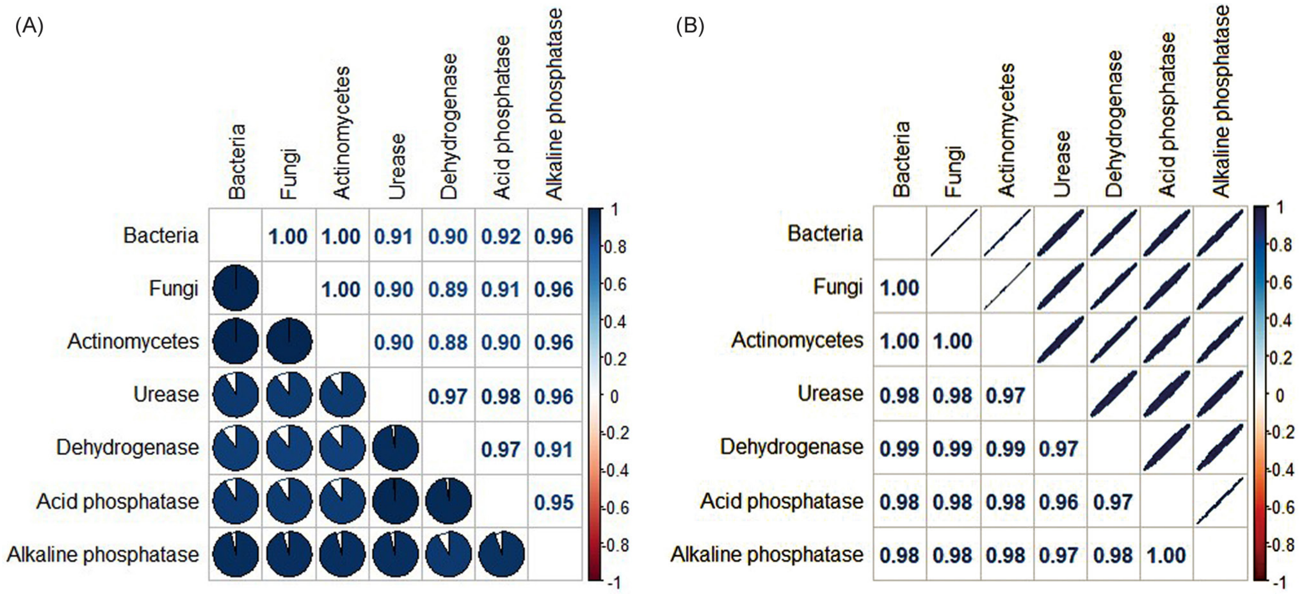

Fig. 2 Correlation coefficient between (A) soil microbial population and (B) soil enzymes activity.

Correlation analysis: Correlation analysis was carried out for soil microbial load and soil enzymes activity under aerobic rice condition. Results showed that soil microbial population observed were highly positively correlated with soil enzymes activity (Fig. 2 A and B).

Thus, it inferred that the incorporation of *dhaincha*, application of half dose of RDN from inorganic fertilizer + quarter dose of RDN from EFYM + quarter dose of RDN from VC + AM fungi + foliar nutrients of 0.5% urea + 1% $FeSO_4$ + 0.5% $ZnSO_4$ (M_2S_6) had significant impact on soil microbial load and soil enzymes activity which resulted in increased growth and yield attributes for higher grain and straw yield of aerobic rice.

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