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NINETEEN-YEAR ASSESSMENT OF NUTRIENT MANAGEMENT ON SOIL MICROBIOLOGICAL AND ENZYMATIC ACTIVITIES OF INCEPTISOLS IN RICE-WHEAT SYSTEM

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ABSTRACT

The soil biological properties and productivity of the rice-wheat system were examined after the long-term (19 years) application of chemical fertilizers (100% N, 100% NP, 100% NPK, and 150% NPK) alone and in combination with organic manures (100% NPK+Straw Incorporation (SI), 100% NPK+Green manure (GM), 100% NPK+Farmyard manure (FYM)). The integrated use of inorganic fertilizers and organic manure enhanced rice and wheat grain production by 5-20% and 12-15%, respectively, compared to balanced inorganic fertilization, and by 115% and 250% over the control (without fertilization). Long-term use of 100% NPK+FYM significantly improved the viable count of aerobic bacteria, fungi, actinobacteria, P-solubilizer, pseudomonads, free-living N-fixers, nitrate reducers, and ammonia oxidizers over the unfertilized control. Integrated usage of chemical fertilizer and organic manure increased the activity of soil enzymes such as dehydrogenase, alkaline phosphatase, urease, and protease. Significant relationships were found between the productivity of rice and wheat and soil biological factors such as soil microbial population (P-solubilizer, free-living N-fixer, pseudomonas, aerobic bacteria) and soil enzyme activities (dehydrogenase activity and microbial biomass carbon). The results showed that long-term application of chemical fertilizer and organic manure increased rice-wheat productivity via improving soil microbiological properties and biological functioning.

Keywords: Crop yield, Enzyme activities, Microbial properties, Long-term, Rice-wheat

Rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) are important staple foods in South Asia and cover over 13.5 million hectares, or about 33% of the total rice area and 42% of the total wheat land (Tripathi *et al*., 2015) in India, Pakistan, and Bangladesh. Rice-wheat system in the Indo-Gangetic Plains (IGP) of South Asia provides food for around one-fifth of the world's population (Saharawat *et al*., 2010). It is a highly nutrient-exhaustive system that removes about 650 kg ha^{-1} of N, P, K, and 0.5-1.0 kg ha^{-1} Zn annually (Shah *et al*., 2011), causing a substantial increase in the need for fertilizers for growing these crops. Despite the country's rising fertilizer usage, numerous regions using the rice-wheat cropping system have noticed a decline in the productivity of both rice and wheat (Sharma *et al*., 2019). The over-exploitation and improper management of soil, the imbalanced application of fertilizers, and the deterioration in the physical, chemical, and biological conditions of the soil are the leading causes of stagnation and decreasing yield trends in the rice-wheat cropping system in Asia (Bhatt *et al*., 2019; Kumar *et al*., 2019). The declining productivity of the rice-wheat system is a serious scientific challenge to sustain the system in India and other nearby nations. The sustainable management of soil helps sustainable crop productivity because crop types and production technologies influence the fertility and edaphic characteristics of the soil under cultivation.

The biological properties of the soil, such as the microbial population and enzymatic activities affecting nutrient cycling and soil organic matter content, can influence crop yield. The decomposition of soil organic matter (SOM), nitrogen fixation, and nutrient mobilisation are all crucial processes aided by soil microbes (Shah *et al*., 2020). The mineralization of organically bound N, P, and S by microbes determines their availability in the soil (Dehsheikh *et al*., 2020). Soil enzymes regulate the biochemical functioning of the soil system, which results in enhanced soil health and crop productivity (Basu *et al*., 2021). Soil processes like nutrient transformation, soil organic carbon lability, and soil

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microbial activity are defined in relation to enzyme activities. Soil microbial biomass carbon (MBC) can be used as an indicator of the transient nutrient pool and to assess the rate of SOM mineralization. The efficiency of the applied chemical fertilizer is also influenced by soil microorganisms but the continuous use of these fertilizers declined soil health (Pahalvi *et al.*, 2021) due to decline in SOM. Integrated use of inorganic fertilizers and organic manures has the potential to enhance the biological properties of the soil through the changes in the microbial population and sustain the availability of nutrients (Devi *et al*., 2017). These changes in microbes are also anticipated to affect soil enzyme activities (Datt and Singh, 2019). The microbial population, MBC, and enzyme activity are the important soil biological characteristics used to study the relationship between soil types, crop management techniques and the health of ecosystems (Sihi *et al*., 2017).

Researchers have seen positive benefits of applying organic manure on soil biochemical and enzyme activity (Yang *et al*., 2019), as a result of accelerated plant growth and root enzyme secretion (Lynch and Panting, 1980). Therefore, it is vital to comprehend the dynamics and state of soil biological and biochemical features for nutrient cycling and soil health evaluation in various ecological situations as well as soil and crop management approaches. The impact of long-term fertilizer application on soil health in various crops has primarily been evaluated in terms of soil chemical and physical properties but with scanty knowledge of soil biological qualities. The objective of this study was to determine the effects of long-term chemical fertilizer and organic manure on soil microbiological and enzyme activities associated with the productivity of the rice-wheat cropping system in order to develop strategic management options in inceptisols. Inceptisols are relatively high in fertility, and play a crucial role in crop production, particularly in regions where intensive farming is practiced.

MATERIALS AND METHODS

Experimental design and treatment details

The present study was conducted from an ongoing long-term fertilizer experiment (since 1999) on a rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) cropping sequence at Punjab Agricultural University, Punjab, India. Geographically, the experimental site is situated at an altitude of 274 m above the mean sea level with latitude 30° 54' 27" N and longitude 75° 46' 59" E. The experimental region is semiarid sub-tropical with an average annual rainfall of approximately 670 mm, with most of the rain falling between July and September. The soils of the experimental sites are alluvial with an order of inceptisols. In 1999, at the start of the experiment, the soil had the following characteristics: pH of 8.3, electrical conductivity of 0.20 dS m^{-1} in a soil-to-water ratio of 1:2.5, soil organic carbon of 2.42 g kg^{-1}, $NaHCO_3$-extractable P of 12.5 kg ha^{-1}, NH_4OAc-extractable K of 85.4 kg ha^{-1}, and a loamy sand texture with 73.2% sand, 21.9% silt, and 4.9% clay. The fertilizer treatments compared in the present study were control, 100% N, 100% NP, 100% NPK, 150% NPK, 100% NPK + straw incorporation (SI), 100% NPK + green manuring (GM), and 100% NPK+ farmyard manure (FYM). Imbalanced fertilization treatments include 100% N, 100% NP and 150% NPK. Urea, single superphosphate and muriate of potash were the fertilizer sources of N, P, and K, respectively. The full dose of P and K was drilled at the time of sowing rice and wheat. Nitrogen was applied in three splits (1/3rd at transplanting, 1/3rd after three weeks of transplanting, and 1/3rd at six weeks of transplanting) in rice and two splits (1/2 at sowing and 1/2 at first irrigation) in wheat. Farmyard manure with mean nutrient content of 350 g kg^{-1} C, 5 g kg^{-1} total N, 2.5 g kg^{-1} total P, and 15 g kg^{-1} total K was applied once in the cropping cycle at a rate of 10,000 kg ha^{-1}, i.e. three weeks before sowing of rice. Green manuring was executed by sowing *Sesbania* spp. after harvesting the wheat. Whole *Sesbania* plants [2.0:0.7:0.5 (N:P:K)] were incorporated into the soil by discing after 45 days of sowing. Annually, wheat (0.47:0.39:0.89) and rice straw (0.49:0.07:0.26) of about 80% were incorporated after harvesting wheat and rice, respectively. Each triplicate plot treatment was laid out in a randomized complete block design (RCBD) of 108 m^2 (12 × 9 m) in size. The crop was raised following the practices recommended by Punjab Agricultural University in the region under irrigated conditions. Rice seedlings were transplanted, and wheat was seeded in the last week of June and November, respectively. The crops were harvested manually, and the grain yield was recorded.

Soil sampling and analysis

Composite surface (0-15 cm) soil samples were collected from each plot, i.e., four random core samples from each plot were thoroughly mixed together at the end of the rice-wheat cropping cycle. The composite samples were placed in plastic

Table 1. Methods used for different biological parameters

Biological parameters	Method	Reference
Population of aerobic bacteria (BACT), fungi, actinobacteria (ACTINO), P-solubilize (PSB)r, pseudomonads (PSED, free-living N-fixers (NF), nitrate reducers (NR) and ammonia oxidizers (AMO)	Serial dilution spread plating technique	Beijerinck (1888)
Soil microbial biomass C (MBC)	Chloroform fumigation extraction	Jenkinson and Powlson (1976)
Soil dehydrogenase enzyme activity (DHA)	Monitoring the rate of production of tri-phenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC)	Casida *et al*. (1964)
Alkaline phosphatase enzymes (ALP)	p-nitrophenyl phosphate tetrahydrate solution of pH 11.0	Tabatabai and Bremner (1969)
Protease enzyme activity (PRO)	Tyrosine as substrate	Ladd and Butler (1972)
Urease enzyme activity (URE)	Estimating urea hydrolysis	Bremner and Douglas (1971)

bags and transported to the laboratory, where fresh soil samples were passed through a 2 mm sieve, homogenized, and stored at 4°C. The methods used for analyzing various parameters are given in Table 1.

Statistical analysis

The impact of fertilizer management was compared using a one-way analysis of variance (ANOVA). A correlation study was done between them to see how certain soil microbiological and enzymatic qualities interacted with other parameters. The findings use the significance level of $p \leq 0.05$. Principal Component Analysis (PCA) (Wold, 1987) was applied to the dataset to analyse the correlations between the observed variables and highlight the similarities and differences across samples. The variables that explained less than 50% of the total variability in the data set were eliminated in order to reduce the number of variables in the dataset.

RESULTS AND DISCUSSION

Yield

The grain yield of rice and wheat was significantly influenced by various inorganic fertilizers and organic manure management practices (Fig. 1). The grain yield of rice and wheat varied from 3300 and 1690 kg ha^{-1} under control to 7120 and 5920 kg ha^{-1}, under the integrated application of 100% NPK+FYM, respectively. Compared to imbalanced fertilization (100% NP and 100% N), balanced application of the mineral fertilizer treatment increased rice and wheat grain production by 8% to 20% and 9% to 15%, respectively, and by 80% and 204% over the control. Among the conjunctive use of organic manures with inorganic fertilizers, the maximum rice and wheat grain yield was recorded under 100% NPK+FYM (7120 kg ha^{-1} and 5920 kg ha^{-1}) subsequently by 100% NPK+GM (6530 kg ha^{-1} and 5810 kg ha^{-1}) and minimum in 100% NPK+SI (6220 kg ha^{-1} and 5780 kg ha^{-1}), respectively. The positive impact of FYM on yield attributes may have been brought about by improved macronutrient and micronutrient supply during the growth season. It might be explained by the solubilization action of FYM on plant nutrients, increasing nutrient absorption, particularly NPK and improved soil properties. These outcomes have supported the conclusions of Bhatt *et al*. (2019).

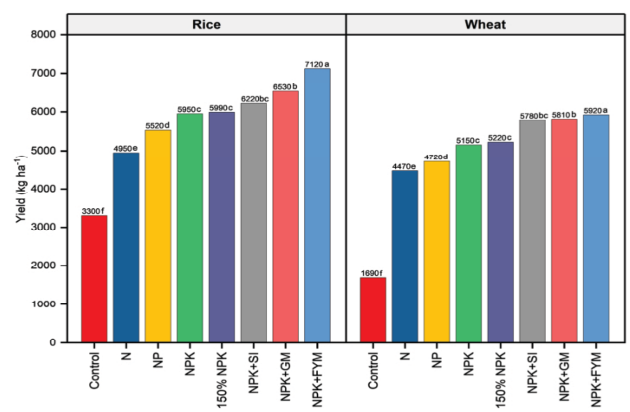

Fig. 1. Effect of long-term application of inorganic and organic fertilizers on the grain yield (kg ha^{-1}) of rice and wheat under rice-wheat system. The different letters in each column graph are significantly different at $p \leq 0.05$ after Duncan's Multiple Range Test (DMRT)

Through microbially mediated nutrient cycling, integrated treatments with FYM increased the microbial species diversity and abundance, providing plants with a more plentiful source of nutrients. It might be because the inclusion of FYM has increased the labile carbon pool, which increased the number of microorganisms. These findings agree with those of Bhattacharyya *et al*. (2013) and Brar *et al*. (2015).

Soil microbial population

The viable count of soil microorganisms was considerably affected by the long-term integration of various nutrient management techniques (Fig. 2). The most abundant populations of aerobic bacteria (8.11 log cfu g^{-1} of soil), fungi (4.76 log cfu g^{-1} of soil), actinobacteria (5.80 log cfu g^{-1} of soil), P-solubilizer (4.86 log cfu g^{-1} of soil), pseudomonads (5.31 log cfu g^{-1} of soil), free-living N-fixer (5.58 log cfu g^{-1} of soil), nitrate reducer (5.28 log cfu g^{-1} of soil) and ammonium oxidizer (4.75 log cfu g^{-1} of soil) were observed in integrated treatment of 100% NPK and FYM. Continuous FYM application over time, together with the balanced chemical fertilizers, resulted in a substantial amount of easily available carbon (C) in the soil, which increased the microbial population relative to chemical fertilizers used alone. Because most soil microorganisms are chemo-heterotrophs, they obtain the C they need to build the cellular components and energy by oxidising the organic materials derived from the soil's organic matter (Coonan *et al*., 2020). Additionally, adding organic matter improves the soil's physical properties, fostering the growth and proliferation of microorganisms (Tejada *et al*., 2009).

The soil microbial population was significantly lower when imbalanced inorganic fertilizers were used (100% N and 100% NP) over 100% NPK. It might be because these treatments have imbalanced amounts of plant nutrients, which has led to a decrease in the production of plant biomass (carbon substrate) (Suresh *et al*., 1999). In terms of the viable counts of the soil microorganisms, the counts for the 150% NPK treatment were comparable to those for the 100% NPK treatment, showing that the supplies of inorganic nutrients at a level above the optimum without organic manure have no effect on the soil microbes and thus could not help in the sustenance of increase in the microbial population in the soil. The results were consistent with the findings of Ingle *et al*. (2014) in Vertisols. However, compared to 100% NPK+FYM, these treatments produced noticeably lower counts, which suggests that organic manure has a greater beneficial effect on the soil microbial community.

Biochemical properties

Soil microbial biomass C

In a long-term fertilizer experiment, organic and inorganic nutrient management techniques

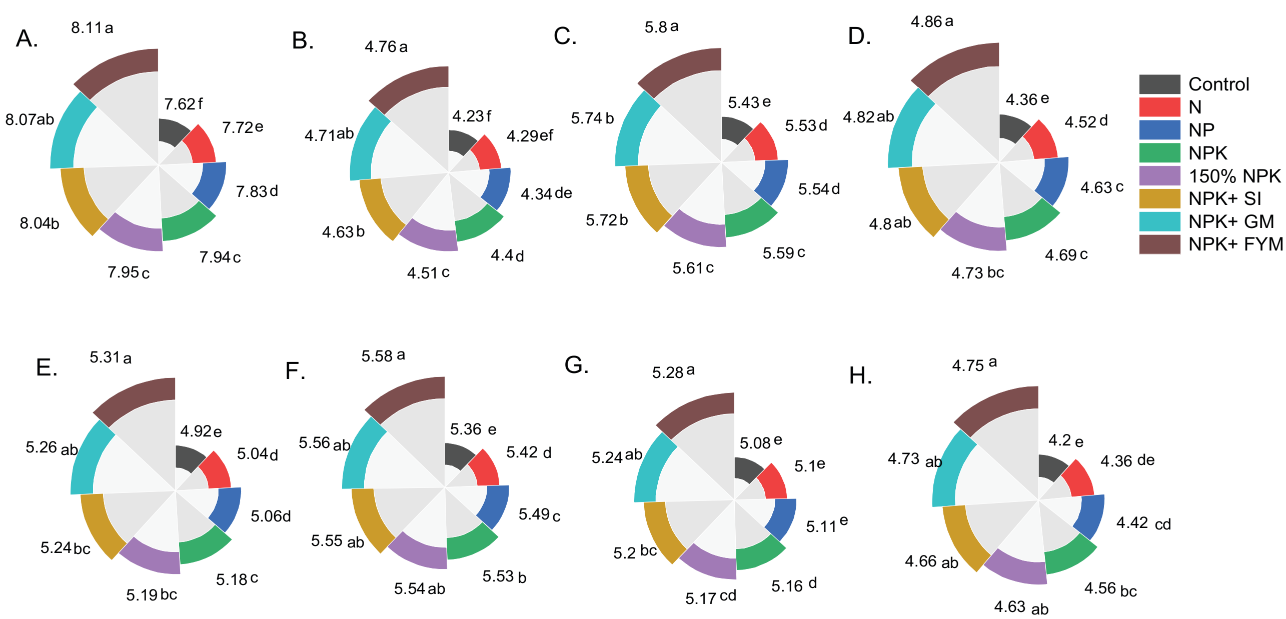

Fig. 2. Population (log cfu g−1 of soil) of A. Aerobic bacteria, B. Fungi, C. Actinobacteria, D. P-solubilizer, E. Pseudomonads, F. Free-living N-fixer, G. Nitrate reducer and H. Ammonium oxidizer in soil under rice-wheat cropping system. The different letters in each pie chart are significantly different at *p≤0.05* after Duncan's Multiple Range Test (DMRT)

considerably impacted soil microbial biomass C (Table 2). The mean soil microbial biomass C increased from 135 mg kg^{-1} in control to 389 mg kg^{-1} in 100% NPK+FYM. This increase in microbial biomass C may be attributed to FYM's catalytic action promoting microbial growth. The use of FYM ensured a balanced supply of nutrients and carbon, which may have assisted in increasing the soil's microbial population (Basak *et al*., 2012). The use of NPK and FYM in Vertisols in Coimbatore has significantly increased soil microbial biomass C, according to Vineela *et al*. (2008).

In comparison to balanced fertilization and the control, the combined application of inorganic fertilizer and organic sources (straw incorporation, green manure, and farmyard manure) increased microbial biomass C by 3% to 33 % and 123% to 188%, respectively. Because of the utilisation of organic resources, which maximises the return of organic matter to the soil and minimises soil disturbance, the MBC increased under integrated nutrient management (Leithold *et al*., 2015). Similar positive effects of combining organic manure and chemical fertilizers on microbial biomass C have been documented by Patil and Puranik (2001). An imbalanced dosage of chemical fertilizers (100% N and 100% NP) led to much lower soil microbial biomass C than the balanced dosage (100% NPK) and super-optimal dosage (150% NPK).

Soil enzymes

Dehydrogenase activity

Dehydrogenase enzyme activity in soil is a common indicator of microbial activity since it reflects the state of overall metabolism. Dehydrogenase enzymes are also transient in the soil system because they are present intracellularly and do not accumulate extracellularly; as a result, this group of enzymes is primarily affected by the addition or degradation of substrate or contaminant and serves as a direct indicator of any modification to the soil microbial community's capacity for C-transformation and metabolic activity (Rozylo and Bohacz, 2020). At the end of the 19^{th} cycle of the rice-wheat cropping system, dehydrogenase activity varied according to the management techniques, with the lowest levels being in the control (3.52 g TPF g^{-1} hr^{-1}) and the highest levels being in 100% NPK+FYM (6.98 g TPF g^{-1} hr^{-1}) (Table 2). In comparison to balanced fertilisation, the integrated application of inorganic fertilizer and organic manure in the forms of SI, GM, and FYM increased dehydrogenase activity by 12% to 16%, and by 91% to 98% over the control. In comparison to all other treatments, the 100% NPK+SI, 100% NPK+GM, and 100% NPK+FYM treatments showed the highest increase in soil dehydrogenase activity. Since C substrates are the only sources of C and energy for heterotrophs, there has been an increase in dehydrogenase activity.

Saha *et al*. (2008) reported a significant increase in dehydrogenase activity in the plots treated with organic manure, especially when combined with NPK. The results are in line with Bhattacharyya *et al*. (2008) findings, which indicated that the addition of FYM along with NPK led to a fourfold increase in soil dehydrogenase activity. These reports suggest that FYM use results in more pronounced biological activity. Earlier studies by Chu *et al*. (2007) found that the impact of organic manure on soil enzyme activity was relatively stronger than that of inorganic fertilizers. Dehydrogenase activity significantly decreased due to imbalanced and insufficient fertilizers (NP and N at 100%).

Soil alkaline phosphatase activity

Alkaline phosphatases have been extensively studied in the soil (Speir and Ross, 1978; Tabatabai, 1994). These enzymes catalyse the hydrolysis of ester-phosphate bonds, releasing phosphate that can be absorbed by plants or microorganisms (Lemanowicz *et al*., 2016). Alkaline phosphatase activity ranged from 3.37 g PNP g^{-1} hr^{-1} in control to 7.00 g PNP g^{-1} hr^{-1} in 100% NPK+FYM (Table 2). Alkaline phosphatase activity increased by 4% to 30% when chemical fertilizer and organic sources (straw, GM, FYM) were applied over balanced fertilisation, whereas it increased by 65% to 107% over control. This difference could be attributed to the additional supply of N and C substrates provided by the applied organic sources for supporting microbial growth. The 100% NPK+FYM had the highest alkaline-phosphatase activity (7.00 g PNP g^{-1} hr^{-1}). The primary cause was that the soil phosphorus availability from organic P-sources was altered by the monophosphoesterase activity, which is directly correlated with alkaline phosphatase activity.

Further, these enzymes' activities are strongly impacted by the type and amount of organic residue incorporation (Imran *et al*., 2020). Alkaline phosphatase activity also increased when organic manure and inorganic fertilizers were used together, according to Garg and Bahl (2008). Numerous investigations have found a correlation between organic matter and alkaline phosphatase activity

(Aon and Colaneri, 2001). Alkaline phosphatase activity could rise by 9% to 29% over imbalanced fertilization and by 60% over control with balanced chemical fertilizer application. Mishra *et al*. (2008) revealed reduced activity of the phosphatase enzymes in the soil as a result of imbalanced usage of chemical fertilizers when reviewing the findings of a long-term study in Alfisol. The growth and activities of microorganisms are encouraged by balanced fertilisation, which supports higher plant biomass production and increases the return of organic residue in the soil through leaf fall and root stubbles.

Soil urease activity

The hydrolysis of urea to carbon dioxide and ammonia by the urease enzyme in the soil is essential for the N cycling. It can build up in the extracellular environment as an exo-enzyme and stabilise by adhering to soil colloids, which have a mixed organo-mineral origin (Mikanova *et al*., 2015). The activity of urease ranged from 251 under control to 350 µg urea hydrolyzed g^{-1} of soil hr^{-1} under 100% NPK+FYM (Table 2). Application of chemical fertilizer along with SI, GM, and FYM treatments increased urease activity by 14% to 17% over balanced fertilization. According to Dilly *et al*. (2007), the addition of organic manure offers enough quantity of organic N that can encourage heterotrophic microorganisms and raise the activity of soil hydrolytic enzymes. Smith and Powlson (2007) reported that easily accessible organic N in manure increased the urease activity. According to Kanchikerimath and Singh (2001), soil organic C and microbial populations were strongly and favourably correlated with soil enzyme activities, particularly soil urease activity. Therefore, integrated nutrient management techniques can increase the activity of the urease enzyme in the soil (Sharma *et al*., 2015).

When chemical fertilizer is applied at the recommended amount (100% NPK), urease activity is enhanced by 5% to 10% over imbalanced fertilisation (100% NP and 100% N) and by 19% over control. An increase in the microbial population and the production of a higher proportion of nitrogenous compounds in root exudates that stimulate urease enzyme activity may be the causes of the favourable effects of an increase in N levels (Elayaraja and Singarvel, 2011).

Soil protease activity

Protein peptide bonds are hydrolyzed by protease, and this is followed by the mineralization of amino acids and nitrogen (Loll and Bollag, 1983). Under the rice-wheat cropping system, application of 100% NPK+FYM resulted in the highest (49.1 g Tyr g^{-1} h^{-1}) protease enzyme activity and the lowest (19.3 g Tyr g^{-1} hr^{-1}) was observed in control (Table 2). Balanced fertilization resulted in 12% to 45% higher protease activity over control, and integrated nutrient management (100% NPK+SI, 100% NPK+GM, and 100% NPK+FYM) enhanced by 96% to 154% over balanced fertilization. The increased protease enzyme activity in the 100% NPK+FYM treatment could be attributable to the increased microbial biomass accumulation brought on by the increased rates of nutrient cycling and organic matter decomposition (Ma *et al*., 2020). Additionally, data showed that peptides serve as the primary source of N for soil microorganisms (Ma *et al*., 2020). The findings suggest that the quicker multiplication of microorganisms, particularly the proteolytic bacteria, was caused by higher mineral nutrients, C, and

Table 2. Influence of different fertilizer treatments on soil microbial biomass carbon, and dehydrogenase, alkaline phosphatase, urease and protease enzyme activities under rice-wheat cropping system

Treatment	MBC (mg kg^{-1})	Dehydrogenase (µg TPF g^{-1} hr^{-1})	Alkaline phosphatase (µg PNP g^{-1} hr^{-1})	Urease (µg urea hydrolysed g^{-1} hr^{-1})	Protease (µg tyr g^{-1} hr^{-1})
Control	135^{g}	3.52^{h}	3.37^{g}	251^{g}	19.3^{g}
100%N	206^{f}	4.46^{g}	4.18^{f}	272^{f}	22.8^{f}
100%NP	240^{e}	5.10^{df}	4.92^{e}	283^{e}	23.1^{f}
100%NPK	292^{d}	6.02^{e}	5.38^{d}	298^{d}	33.8^{e}
150% NPK	299^{c}	6.10^{d}	5.40^{d}	304^{c}	36.3^{d}
100% NPK+ SI	301^{c}	6.73^{c}	5.57^{c}	339^{b}	37.9^{c}
100% NPK+ GM	309^{b}	6.8^{ab}	5.62^{b}	344^{ab}	39.9^{b}
100% NPK+ FYM	389^{a}	6.98^{a}	7.00^{a}	350^{a}	49.1^{a}

Within a column, means followed by different letters differ significantly after DMRT at $p \leq 0.05$

energy sources (Chang *et al*., 2007). As a result of the mineralization and immobilisation processes, Haynes and Beare (1996) also documented the beneficial effect of mineral N buildup, which led to a larger level of substrate N for organic-derived proteolytic bacteria. Organic colloids from the additional manures may have been engaged in preserving and stabilising enzymes, resulting in higher protease activity, in addition to acting as sources of energy and carbon and releasing nutrients (Miller and Dick, 1995).

Principal component analysis (PCA)

The productivity of rice and wheat was substantially connected with soil biological parameters, such as soil microbial population (P-solubilizer, free-living N-fixer, aerobic bacterial, pseudomonas), and soil enzyme activities, at the surface soil layer (0-15 cm) in the rice-wheat cropping system (dehydrogenase activity and microbial biomass carbon) (Figure 3). P-solubilizer population (r = 0.981), microbial biomass C (r = 0.975), free-living N-fixer population (r = 0.973), pseudomonads (r = 0.965), and dehydrogenase activity (r = 0.961) were all substantially linked with rice yield. Similar to this, the P-solubilizer population (r = 0.945), free-living N-fixer population (r = 0.931), dehydrogenase activity (r = 0.922), pseudomonad population (r = 0.916), and aerobic bacteria population (r = 0.901) all substantially linked with wheat yield. The findings of the current study are consistent with those of Choudhary *et al*. (2021), who found that MBC and DHA were strongly correlated with wheat output over a long-term (48 years) integrated fertilizer experiment with soybean-wheat rotation.

The PCA showed that there were significant variations in the usage of different chemical fertilizers, organic amendments, and their combinations between the various microbial communities and soil enzyme activity. In the soil's microbiological qualities under the rice-wheat cropping system, the PCA identified two main components (PC1 and PC2), which together account for 96.22 % and 2.86 %, 95.5 % and 2.67 % of the overall variance. Regarding the microbial population, PCA revealed two unique groups: group 1) NF, PSB, AMO, BACT, and PSED; and group 2) ACTI, NR, and FUNGI (Fig. 4). Additionally, PCA demonstrated that the integrated application of organic manure and inorganic fertilizer, such as 100% NPK+GM and 100% NPK+FYM, is comparable to and has a stronger impact on the group 2 microorganisms than other treatments. Group 1 is closely resembled by a balanced dose (100% NPK), super-optimal dose (150% NPK), and

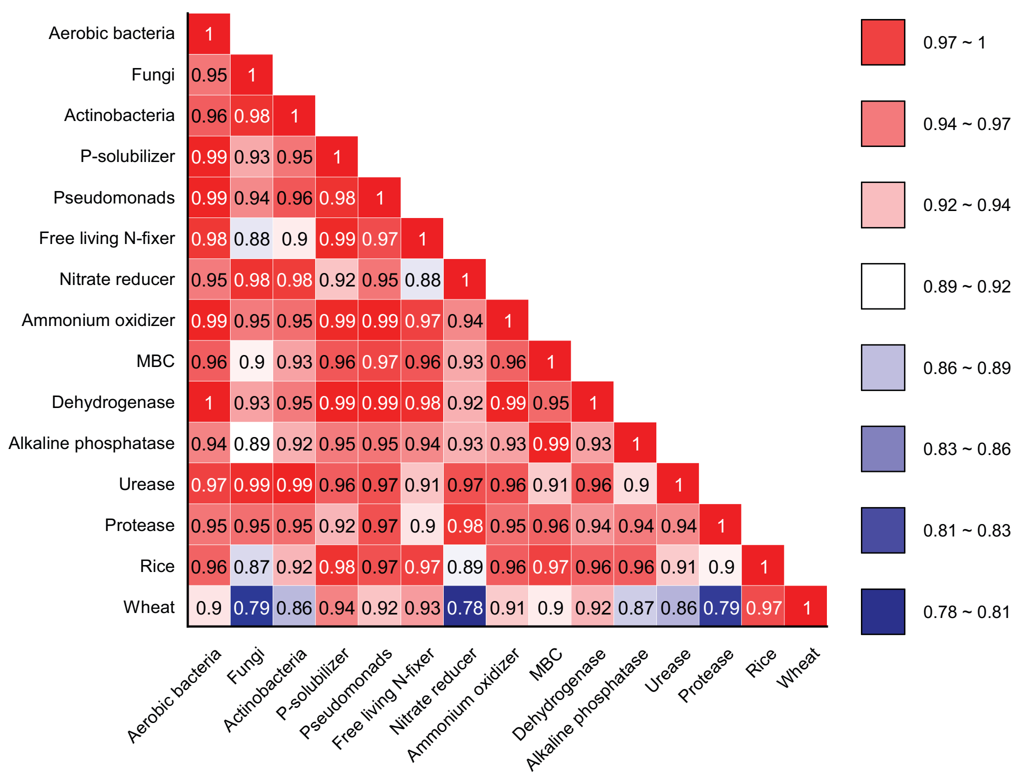

Fig. 3. Correlation among soil microbial population, enzyme activities and grain yield of rice and wheat

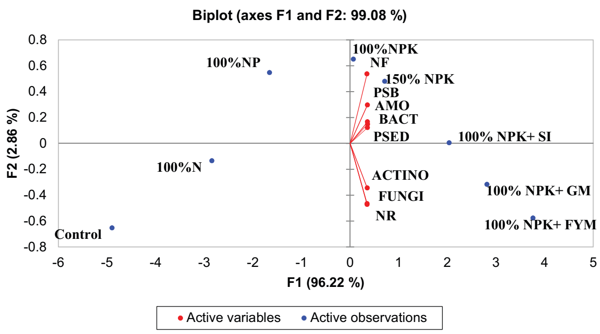

Fig. 4. Principal component analysis (PCA) for microbial population enumeration under rice-wheat cropping system. BACT-Aerobic bacterial population; FUNGI-Fungi population; ACTINO-Actinobacteria population; PSB-P solubilizer population; PSED-Pseudomonads population; NF-Free living nitrogen fixers population; NR-Nitrogen reducer population; AMO-Ammonium oxidizer population

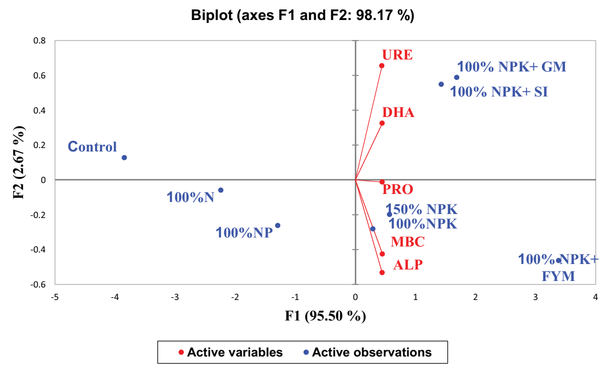

Fig. 5. Principal component analysis (PCA) for soil enzyme activities under rice-wheat cropping system. DHA-Dehydrogenase activity; ALP-Alkaline phosphatase activity; PRO-Protease activity; URE-Urease activity; MBC-Microbial biomass C

integrated application of straw incorporation and inorganic fertilizer (100% NPK+SI).

However, group 1 is more affected by the super-optimal amount of NPK than by a balanced dose of fertilizer, while the other treatments have no impact. Zhang *et al*. (2014) reported that in the factorial space defined by two PCs, PCA distinguished the control treatment from the integrated nutrient management of inorganic fertilizer and organic manure. They noticed that the fungal, actinobacterial, and pseudomonad populations were all clustered together in the orthogonal space, indicating that the integrated nutrient management of chemical fertilizer with organic manure had a greater impact on these parameters than the control treatment.

PCA results for the soil enzyme activity showed the presence of two different groups: group 1) URE, DHA, and group 2) PRO, MBC, and ALP. PCA also demonstrated that green manure (100% NPK+GM) and inorganic fertilizer (100% NPK+SI) are closely related and have a greater impact on group 1 than either alone (Fig. 5). Further, in group 2, the effects of a balanced dose (100% NPK) and a super optimum dose (150% NPK) of inorganic fertilizer are greater than those of a combined application of fertilizer (100% NPK+FYM). The combined use of SI with NPK and GM with NPK for group 1 is close and has a greater impact on group 1. The other treatments had no impact on group 1. According to Tamilselvi *et al*. (2015), assayed variables distinguished between soil that had been managed organically and soil that had been managed inorganically. The majority of the assayed variables, including microbial biomass C, dehydrogenase, urease, and alkaline phosphatase, were found to be associated with soil that had been managed organically, suggesting that the addition of diversified carbon sources through organic manure enhanced microbial biomass and enzymatic activities.

CONCLUSION

The viability of aerobic bacteria, fungi, actinobacteria, P-solubilizers, pseudomonads, free-living N-fixers, nitrate reducers, and ammonia oxidizers is improved with long-term application of 100% NPK + FYM. Applying balanced fertilizer with organic manure together boosted the activity of soil enzymes such as dehydrogenase, alkaline phosphatase, urease, and protease. Significant relationships were found between the productivity of rice and wheat and soil biological factors such as soil microbial population (P-solubilizer, free-living N-fixer, pseudomonas, aerobic bacteria), and soil enzyme activities (dehydrogenase activity and microbial biomass carbon). Long term use of chemical fertilizer and organic manure ehance rice and wheat productivity while having no negative effects on microbiological and biological functioning of the inceptisol.

Authors' contribution

Execution of field/lab experiments and data collection, Analysis of data and wrote the manuscript (SJ); Conceptualization of research work and designing of experiments, and interpretation of data (GSD); Analysis and interpretation of data (AK); Interpretation of results, reviewing and editing of manuscript, and helped shape the manuscript according to the Journal guidelines (GN).

Conflicts of interest

Authors declare that they hve no conflicts of interest.

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