



Original Research Article

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Long-Term Impact of Balanced and Imbalanced Fertilization in Rice under Rice-Wheat Sequence in Mollisols on Relationship between Microbial Activity and Yield

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The impact of the long-term fertilization alone and in combination with FYM for 30 years was evaluated on microbial and enzymatic activities after rice under a continuing long-term fertility experiment in rice-wheat cropping system initiated in *kharif*, 1984 at G.B. Pant University of Agriculture and Technology, Pantnagar. The experiment comprised 14 treatments having absolute control, individual N and its combination with P and K at varying levels alone and with FYM. The balanced application of nutrients through chemical fertilizers alone or with FYM resulted in higher microbial activity in terms of higher dehydrogenase and urease activity in soil; microbial respiration and synthesis of microbial biomass nitrogen in different soil layers through the soil profile up to 60 cm. The higher microbial activity was evident in surface layer (0-15 cm) decreasing with depth in all the treatments. Highest buildup of dehydrogenase and urease activity, microbial respiration and microbial biomass nitrogen in all the layers up to 60 cm depth was supported by the application of $N_{180} + P_{80} + K_{40} + Zn(F) + FYM$ except microbial biomass nitrogen (MBN) in sub-surface layers (15-30 and 30-60 cm). The MBN synthesis was higher in sub-surface layers due to $N_{120} + P_{40} + K_{40} + ZnF + FYM$. Maximum grain yield was also produced due to application of $N_{120} + P_{40} + K_{40} + Zn (F) + FYM$. The significant strong correlations were observed between MBN, dehydrogenase and urease activity; microbial respiration and grain yield of rice. The results indicated that long-term addition of balanced fertilizer in conjunction with FYM has the most beneficial effects on soil biological health *vis-à-vis* rice productivity.

Introduction

Soil management practices such as tillage, crop rotation, green manuring, and nutrient management practices influence the soil's capability to support the productivity of the crops. In general these practices directly or indirectly affect the biological soil health *vis-à-vis* nutrient supply to the crops. Soil

microorganisms are essential for the long-term sustainability of agricultural systems (Wardle *et al.*, 1999) as they mediate the biological processes influencing the organic matter decomposition, humus formation, and nutrient cycling through enzymatic activities (Smith and Paul, 1990; Sinsabaugh *et al.*,

1991). Soil enzymes are pivotal components involved in the dynamics of soil nutrient transformations (Masto *et al.*, 2006). Enzyme activity in the soil environment is considered to be the major contributor of overall soil microbial activity and soil quality (Roldan *et al.*, 2005). Soil enzymes occur as exo- and endoenzymes present in viable microbial cells and are a part of the soil matrix (Dick *et al.*, 1994). Soil enzyme activities are commonly correlated with microbial parameters thus can be a sensitive index to evaluate the long-term management effects of organic and green manures; tillage (Deng and Tabatabai, 1997; Dick *et al.*, 1988; Kirchner *et al.*, 1993) and chemical fertilizers.

Biological soil quality can also be judged by measuring the soil microbial biomass and microbial activity as have been considered as important indices (Schloter *et al.*, 2003). The soil biological parameters respond quickly in a year or two to crop and soil management practices (Livia *et al.*, 2005). The application of nutrients inadequately and/ or imbalanced use of fertilizer with meager or no use of organic manures deteriorates the productivity of soil leading to the poor soil quality because of low biomass production.

In developing world the nutrients are not provided adequately for the crop production being costlier and poor purchasing power of farmers leading to deterioration of soil quality and unsustainability of soils. Rice-wheat is a dominant cropping system and it occupies 13.5 m ha in the Indo-Gangetic Plains of Bangladesh, India, Nepal, Pakistan (Ladha *et al.*, 2000). Long-term fertilizer experiments are vital tools to assess management induced variability on sustainability and processes occurring in soil during nutrients turnover. Long-term application of chemical and organic amendments significantly influences the quality and productivity of soil, mainly through improving nutrient availability to

plants and microorganisms (Acton and Gregorich., 1995). Both chemical and organic amendments can maintain or improve crop yields, and their application can directly or indirectly causes changes in soil chemical, physical and biological processes *vis-à-vis* properties (Belay *et al.*, 2002). Microbial respiration can also be employed as an index of biological activity of soil as it depicts the physiological efficiency of soil microbes.

Therefore, the present investigation was carried out with the hypothesis that balanced and imbalanced fertilization with or without FYM in rice-wheat system in Mollisols for a long-term will have differential impact on enzymatic activities, microbial activity and their relation to yield of rice. The outcome of the study will be helpful to pinpoint the nutrient management practice(s) for rice sustainability under rice-wheat system in Indo-Gangetic Plains (IGP) to keep the soil sustainable and healthy.

Materials and Methods

Experimental site and soil characteristics

The long-term experiment is continuing in A₂ block of Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, (Uttarakhand), which lies in a narrow belt under the foothills of the Shivalik range of Himalayas since 1984. The experimental site falls in the sub-humid, sub-tropical climate zones locally known as the *Tarai* region, situated at 29°N latitude and 79°3'E longitude and at an altitude of 243.84 metres above mean sea level in Uttarakhand. The *Tarai* area has a dry season from early October to mid-June, and a wet season from mid-June to early October. Temperatures are highest in May-June and lowest in December-January. Relative humidity is highest in July-August and lowest in April-May. Total rainfall

ranges from 1300-1500 mm per annum of which about 85-90% is received from June to September.

The soil of the experimental plot is alluvial in origin, silty loam in texture, relatively young and is classified as Aquic Hapludoll. The initial soil properties recorded in 1984 for 0-15 cm depth are given in table 1.

Experimental design and treatments

The experiment has been laid in Randomized Block Design (RBD) with fourteen treatments and four replications (Tables 2 and 3). During *kharif* and *rabi* seasons, rice (cv. Pant Dhan 4) and wheat (cv. UP 2425) were taken as test crops at a fixed site with the same layout plan.

Soil Sampling and biological parameters

After completion of the 30th cycle of rice in 2014-15, representative soil samples from 0-15, 15-30 and 30-60 cm depth were collected. The samples were stored at 0-40 C for the assessment of biological properties and were exposed within 20-30 days of sampling.

Dehydrogenase activity was measured by the method listed by Casida *et al.*, (1964) and urease activity employing the method of Pal *et al.*, (1981). The microbial respiration was recorded by the alkali trap method (Anderson, 1982). Microbial biomass nitrogen in soil samples was estimated by chloroform fumigation and extraction method as described by Brookes *et al.*, (1985) and modified by Vance *et al.*, (1987) and nitrogen in fumigated and non-fumigated sets was determined by Kjeldahl digestion-distillation-titration method (Black, 1965). The grain yield of rice was recorded after the harvest of crop.

Statistical analyses

The data was processed as per the procedure of R.B.D (Snedecor and Cochran, 1967),

using STPR software of G.B.P.U.A. & T., Pantnagar. Correlation coefficients were computed using SPSS programme (SPSS, 1990).

Results and Discussion

Biological indices

Various biological indices of soil quality were significantly influenced due to the balanced and imbalanced nutrient supply through various levels of chemical fertilizers alone and with FYM in rice under rice-wheat crop rotation in Mollisols for 30 years.

Dehydrogenase activity

Dehydrogenase activity in surface layer (0-15 cm) increased significantly due to application of all fertilizer combinations alone and with FYM for 30 years except N₁₂₀ and N₁₂₀ + K₄₀. Application of N₁₈₀ + P₈₀ + K₄₀ + Zn (F) + FYM supported highest dehydrogenase activity which was significantly higher than all other treatments. In 15-30 cm and 30-60 cm soil layer too, application of N₁₈₀ + P₈₀ + K₄₀ + Zn(F) + FYM resulted in highest dehydrogenase activity of 174.58 and 125.97 µg TPF 24 h⁻¹ g⁻¹, respectively (Table 2). The enhanced dehydrogenase activity in soil receiving balanced fertilization along with FYM could be due to improvement in soil organic carbon, total N and P status through incorporated FYM, enhanced root biomass, and rhizodeposition that enhanced the microbial proliferation leading to stimulated microbial activity and organic matter degradation *vis-a-vis* dehydrogenase activity in soil. Organic and integrated nutrient supply (FYM; NP + FYM; NPK + FYM) supported the highest dehydrogenase activity (Srinivas *et al.*, 2015; Bhatt *et al.*, 2016; Liu *et al.*, 2010). The enhanced dehydrogenase activity was due to higher organic matter content and relatively higher microbial activity as

indicated by higher MBC. Dehydrogenase activity is influenced by the quality than by the quantity of organic matter incorporated into soil. Thus, the stronger effects of farm yard manure on dehydrogenase activity might be due to the more easily decomposable components of farm yard manure on the metabolism of soil microorganisms (Pancholy and Rice, 1973).

Urease activity

Long-term fertilizer application at varying doses with or without FYM significantly influenced the urease activity at all the depths up to 60 cm. The highest amount of NH₄-N derived through urease activity to the tune of 2.87, 2.17 and 1.14 mg NH₄-N g⁻¹ soil h⁻¹ due to application of N₁₈₀ + P₈₀ + K₄₀ + Zn(F) + FYM for 30 years in 0-15, 15-30 and 30-60 cm, respectively and lowest in the absence of extraneous supply of nutrients i.e. control (Table 2). The beneficial effects of FYM + NPK on soil urease activity may be attributed to higher level of organic carbon and nitrogen that led to greater microbial proliferation in soil *vis-à-vis* greater amount of urease enzyme synthesis. The stimulatory effects of nutrient supply through organic sources on urease activity are evident in the literature (Bhatt *et al.*, 2016; Jadhav *et al.*, 2016; Saha *et al.*, 2008).

Microbial respiration

Microbial respiration or CO₂ evolution represents overall metabolic efficiency in soil thus it is considered as an indicator of microbial activity. Microbial respiration activity ranged from 0.89 to 2.90, 0.69 to 1.91 and 0.13 to 1.10 µg CO₂-C 100 g⁻¹ soil h⁻¹ at 0-15, 15-30, and 30-60 cm soil layer, respectively. The highest activity was supported by N₁₈₀ + P₈₀ + K₄₀ + ZnF + FYM In all the depths of soil profile up to 60 cm (Table 3). The increase in microbial respiration activity in soil, due to addition of

FYM along with inorganic fertilizers can be ascribed to enhanced availability of C from FYM, root biomass and rhizodeposition which act as an energy source for micro-organisms native to the soil and FYM. The enhanced microbial respiration indicates the increased microbial activity, leading to enhanced mineralization and consequent release of CO₂. Liu, *et al.*, (2010) recorded the highest microbial respiration in soil (6.01 µg CO₂-C g⁻¹ day⁻¹) under NP + FYM treatment followed by FYM treatment and lowest (3.99 µg CO₂-C g⁻¹ day⁻¹) in control. These results also corroborate with the findings of Bhatt *et al.*, (2016), they reported the maximum and significantly higher CO₂ evolution with 100% NPK + 15 t FYM ha⁻¹ over all other treatments, after both rice and wheat crops, was evident.

Microbial biomass nitrogen

The synthesis of microbial biomass nitrogen in surface layer (0-15 cm) was significantly influenced due to varying doses of fertilizer application alone or in combination with FYM over control except N₁₂₀ and N₁₂₀ + K₄₀. The highest microbial biomass nitrogen synthesis (42.78 µg g⁻¹) was supported by application of N₁₈₀ + P₈₀ + K₄₀ + Zn (F) + FYM, in 0-15 cm layer. While in 15-30 and 30-60 cm layer greatest microbial biomass nitrogen was synthesized due to N₁₂₀ + P₄₀ + K₄₀ + Zn (F) + FYM followed by N₁₈₀ + P₄₀ + K₄₀ + Zn (F) + FYM which were at par with each other while lowest MBN was registered in control (Table 3). The lower amount of MBN in control could be attributed to low amount of substrate/food for microbes. High soil organic carbon, more root incorporation and additional supply of organic substrate through FYM for the microorganisms supported the improved microbial biomass nitrogen. Microbial biomass in soil is dynamic and responds to crop input such as fertilization (McGill *et al.*, 1986, Garcia and Rice, 1994). Liu *et al.*, (2010) measured the

highest microbial biomass nitrogen (56.2 mg kg^{-1}) in soil under NP + FYM treatment followed by FYM treatment and lowest (17 mg kg^{-1}) in control.

Grain yield

Grain yield of rice varied from 2118 to 6825 kg ha^{-1} with the application of various fertilizer with or without FYM and effects produced due to different combination of fertilizer application were statistically significant in all the treatments over control. Maximum grain yield was recorded with $\text{N}_{120} + \text{P}_{40} + \text{K}_{40} + \text{Zn} (\text{F}) + \text{FYM}$ which was at par with $\text{N}_{120} + \text{P}_{40} + \text{K}_{40}$ and $\text{N}_{180} + \text{P}_{40} + \text{K}_{40} + \text{Zn} (\text{F}) + \text{FYM}$ and minimum in control (Table 3). The increase in grain yield of rice due to addition of organics through FYM may be attributed to supply of N, P, K and micronutrients in addition to the recommended dose of fertilizers and increase in total N, P and K uptake. An application of FYM also acts a source of substrate for microbial metabolism resulting in enhanced microbial activity and subsequently nutrient transformations resulting in enhanced plant growth and yield. Thind *et al.*, (2016) reported increase in N, P and K uptake to the tune of 29 to 32 %, 29 to 33 % and 27 to 49 %

in wheat due to FYM which increased grain and straw yield. The variation in increasing crop yield under the organic inputs might be attributed to the composition of organic materials since carbon and nitrogen mineralization are largely influenced by the quality of plant materials. The integrated supply of nutrients found beneficial in recording higher grain and straw yield of rice.

Relationship between enzymatic activities, microbial respiration, microbial biomass nitrogen and yield

Correlations studies showed a significant relationship ($P=0.01$) among soil enzymes, microbial respiration, microbial biomass nitrogen and yield in all the three soil depths (Table 4). Correlation coefficients were found dissimilar between above parameters in three soil depths. This indicates differential microbial activities as well as addition of nutrients under long-term fertilizer and FYM application. In all the three depths the MBN found to be strongly correlated with DHA and UA which indicates that the enzymes are of microbial origin. The strong relationship between MBN and MR depicts the higher metabolism of microbes in the presence of adequate supply of nutrients.

Table.1 Soil properties of experimental soil initially in 1984

Parameters	Values
Textural class	Silty loam
pH (1 : 2.5 soil water)	8.0
EC (dS m^{-1})	0.12
Bulk density (g cm^{-3})	1.33
CEC (meq 100 g^{-1} soil)	20.0
Organic matter (%)	2.1
Total nitrogen (%)	0.1
Available Phosphorus (kg P ha^{-1})	20.0
Available potassium (kg K ha^{-1})	222.0
Available Zn (ppm)	0.8

Table.2 Impact of long-term balanced and imbalanced fertilization for 30 years in Rice-Wheat cropping system on dehydrogenase Activity (DHA) and urease activity in 0-60 cm profile after rice

Treatments	DHA ($\mu\text{g TPF } 24 \text{ h}^{-1} \text{ g}^{-1}$ soil)			Urease activity ($\text{mg NH}_4\text{-N g}^{-1} \text{ h}^{-1}$)		
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm
T₁ Control	154.08	126.82	15.70	1.56	1.09	0.22
T₂ N ₁₂₀	166.68	133.66	24.12	1.59	1.17	0.29
T₃ N ₁₂₀ + P ₄₀	216.52	140.00	58.06	1.83	1.40	0.45
T₄ P ₄₀ + K ₄₀	201.32	136.68	52.43	1.71	1.36	0.37
T₅ N ₁₂₀ + K ₄₀	169.16	136.13	38.52	1.67	1.28	0.33
T₆ N ₁₂₀ + P ₄₀ + K ₄₀	225.20	144.20	70.18	2.18	1.71	0.60
T₇ N ₁₂₀ + P ₄₀ + K ₄₀ + Zn(F)	229.71	152.73	74.90	2.33	1.78	0.84
T₈ N ₁₂₀ + P ₄₀ + K ₄₀ + FYM	247.64	164.37	100.11	2.45	2.05	1.11
T₉ N ₁₂₀ + P ₄₀ + K ₄₀ + Zn(F) + FYM	251.48	173.74	117.50	2.64	2.09	1.12
T₁₀ N ₁₈₀ + P ₈₀ + K ₄₀ + Zn(F) + FYM	290.37	174.58	125.97	2.87	2.17	1.14
T₁₁ N ₁₅₀ + P ₈₀ + K ₄₀	232.28	159.14	92.97	2.37	1.82	0.87
T₁₂ N ₁₈₀ + P ₈₀ + K ₄₀ + Zn(F)	226.91	145.48	71.69	2.21	1.74	0.72
T₁₃ N ₁₈₀ + P ₈₀ + Zn(F)	237.04	160.71	96.86	2.44	1.98	1.07
T₁₄ N ₁₂₀ + P ₄₀ + K ₄₀ (DAP)	223.65	143.85	63.13	2.10	1.59	0.55
S.Em±	12.38	6.08	3.66	0.09	0.05	0.04
C.D. at 5%	36.18	17.76	10.68	0.26	0.15	0.12

Zn (F)-Foliar Zinc (0.5% ZnSO₄ + 0.25% Slaked lime); FYM- Farm Yard Manure applied @ 5 t ha⁻¹ on dry weight basis

Table.3 Impact of long-term balanced and imbalanced fertilization for 30 years in Rice-Wheat cropping system on microbial Respiration, microbial biomass nitrogen (MBN) in 0-60 cm profile after rice and grain yield of rice

Treatments	Microbial respiration ($\mu\text{g CO}_2\text{-C } 100 \text{ g}^{-1} \text{ h}^{-1}$)			MBN ($\mu\text{g g}^{-1} \text{ soil}$)			Grain Yield (kg ha^{-1})
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	
T₁ Control	0.89	0.69	0.13	26.51	18.57	9.81	2118
T₂ N ₁₂₀	0.92	0.71	0.20	27.76	20.82	11.10	3024
T₃ N ₁₂₀ + P ₄₀	1.78	1.25	0.40	29.84	23.35	13.19	5102
T₄ P ₄₀ + K ₄₀	1.58	1.19	0.33	29.29	22.90	12.63	3080
T₅ N ₁₂₀ + K ₄₀	1.12	0.88	0.26	28.59	21.65	11.94	2917
T₆ N ₁₂₀ + P ₄₀ + K ₄₀	1.91	1.32	0.49	34.14	27.48	17.77	5399
T₇ N ₁₂₀ + P ₄₀ + K ₄₀ + Zn(F)	2.05	1.42	0.62	35.67	28.73	19.02	5696
T₈ N ₁₂₀ + P ₄₀ + K ₄₀ + FYM	2.57	1.45	1.02	40.25	34.31	23.60	6809
T₉ N ₁₂₀ + P ₄₀ + K ₄₀ + Zn(F) + FYM	2.71	1.58	1.08	41.22	37.84	28.12	6825
T₁₀ N ₁₈₀ + P ₈₀ + K ₄₀ + Zn(F) + FYM	2.90	1.91	1.10	42.78	36.28	26.57	6781
T₁₁ N ₁₅₀ + P ₈₀ + K ₄₀	2.11	1.45	0.76	36.23	29.29	19.57	6094
T₁₂ N ₁₈₀ + P ₈₀ + K ₄₀ + Zn(F)	1.98	1.39	0.53	34.42	28.20	18.49	5714
T₁₃ N ₁₈₀ + P ₈₀ + Zn(F)	2.44	1.44	0.86	36.39	31.45	20.74	6292
T₁₄ N ₁₂₀ + P ₄₀ + K ₄₀ (DAP)	1.85	1.28	0.46	33.31	26.37	17.66	5472
S.Em±	0.04	0.03	0.03	0.89	0.86	1.10	140.51
C.D. at 5%	0.12	0.09	0.07	2.61	2.52	3.22	403.44

Zn (F)-Foliar Zinc (0.5% ZnSO₄ + 0.25% Slaked lime); FYM- Farm Yard Manure applied @ 5 t ha⁻¹ on dry weight basis

Table.4 Correlations between dehydrogenase activity (DHA), urease activity (UA), microbial respiration (MR), microbial biomass nitrogen (MBN) and grain yield (GY) of rice in 0-60 cm profile

	0-15 cm				15-30 cm				30-60 cm			
	DHA	UA	MR	MBN	DHA	UA	MR	MBN	DHA	UA	MR	MBN
UA	0.950 ^{**}				0.962 ^{**}				0.962 ^{**}			
MR	0.975 ^{**}	0.963 ^{**}			0.890 ^{**}	0.931 ^{**}			0.978 ^{**}	0.983 ^{**}		
MBN	0.939 ^{**}	0.984 ^{**}	0.964 ^{**}		0.980 ^{**}	0.981 ^{**}	0.900 ^{**}		0.966 ^{**}	0.958 ^{**}	0.972 ^{**}	
GY	0.934 ^{**}	0.936 ^{**}	0.948 ^{**}	0.925 ^{**}	0.895 ^{**}	0.950 ^{**}	0.910 ^{**}	0.921 ^{**}	0.931 ^{**}	0.926 ^{**}	0.907 ^{**}	0.915 ^{**}

**: Significance at 0.01 level

Similarly, GY showed a strong positive relationship with MBN, DHA, UA and MR representing the increase in yield with increase in enzymatic and microbial activities. Significant correlations of GY with MR, MBN and enzyme assessed implies the importance of organic inputs in nutrient cycling as well as improving soil quality (Nayak *et al.*, 2007, Liu *et al.*, 2009). At 0-15 cm soil layer, significant correlations were observed between DHA and GY ($r=0.934^{**}$); UA and GY ($r=0.936^{**}$); MBN and GY ($r=0.925^{**}$) and; MR and GY ($r=0.948^{**}$). Similar trend of the relationship between these parameters was noticed at 15-30 and 30-60 Cm depths (Table 4).

The close relationship between enzymatic activities with SOC content might be due to the promoting effects of SOC on MBC, MBN and enzyme activities (Baligar *et al.*, 1999). Sangma, *et al.*, (2016) observed the positive correlations between MBN and DHA ($r=0.57^{**}$), SR and MBN ($r=0.38$) and Nath *et al.*, (2012) reported a significant correlations of rice yield with DHA activity ($r = 0.77^{**}$). Significant correlation between the enzymes exhibited the prevalence of intracellular and extracellular enzymes in paddy soil that offer steady biological activity and soil functionality under organic amendments.

After 30 years of experiment in Mollisols of Pantnagar (India), continuous application of balanced inorganic fertilizer alone or in

conjunction with farm yard manure has a significant effect on soil respiration, microbial biomass nitrogen and enzymatic activities i.e. dehydrogenase and urease activity; and grain yields of rice. Application of recommended doses of NPK and FYM improved the soil quality parameters which in turn supported better crop productivity. The enhanced rice grain yield due to balanced supply of nutrients through chemical fertilizers and FYM are supported through significant positive correlations between MBN, enzymes, MR and grain yield. Thus, the study concludes the importance of balanced fertilization and addition of FYM to the soil for successful soil quality management. The adoption of these recommendations in the field will ensure the sustainability of rice production through maintenance/ improvement in the biological soil quality. These practices to farmers to minimize the input costs of production and improve agriculture economy.

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