Journal of the Indian Society of Soil Science, Vol. 66, No. 2, pp 208-214 (2018)
DOI: 10.5958/0974-0228.2018.00026.9

Evaluation of Rice-Rice System on Grain Yield, Chemical, and Biological Properties of an Acid Inceptisols

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Soil microbial population, microbial biomass carbon (MBC), enzyme activities, available nitrogen (N), phosphorus (P) and potassium (K) contents were evaluated in an acidic Inceptisols (udic Ustochrept) after five crop cycles of rice-rice system, fertilized with mineral fertilizer alone or in combination with farmyard manure (FYM). The studied long-term manured treatments included 100% N, 100% NP, 100% NPK, 100% NPK+FYM (5 t ha^{-1}) and an unmanured control. Soils of fertilized plots showed a higher microbial activity in terms of microbial population, MBC and enzymic activities than those under unfertilized control which exihibited a fall from the initial value of the experiment. The microbial activity in the fertilized plots decreased in the order of NPK+FYM > NP > N > NPK. Lower activity in 100% NPK than 100% NP demonstrated the negative impact of K and higher activity in 100% NP treatment than 100% N showed the positive impact of P on microbial activity. Between N and P, the effect of former was more pronounced than that of the latter. Highest activity in 100% NPK+FYM treatment was due to the high positive effect of FYM that overrode the negative effect of K. Highest soil organic matter with favourable pH and available N, P and K were recorded under integrated use of FYM and chemical fertilizers. Continuously unmanured soils showed a very low levels of available N, P and K where N and P levels measured lower values than the initial levels. The soils with higher microbial activity recorded higher yield and were more sustainable.

Key words: Long-term fertilizer treatment, manure, microbial biomass carbon, microbial population, sustainability, soil fertility

Enhancing productivity of rice is the prime concern for all of us in a situation where population growth is showing an ever increasing trend. Long-term studies both in India and abroad have vividly demonstrated that indiscriminate use of nutrients exhibited adverse effect on crop productivity (Sharma *et al*. 2007). Application of organic manures also improves the physical and microbial condition of the soil and enhances fertilizer use efficiency when applied in conjunction with mineral fertilizer (Babu *et al*. 2007). Soil productivity and sustainability are intimately linked to soil biological properties, which provide resiliency and buffering capacity of soil to alleviate stress. Maintenance of soil fertility is essential to improve and sustain crop yield and soil organic carbon (SOC) (Stark *et al*. 2007). In recent years, indicative components like soil microbial biomass carbon (MBC), community structures, functions, and enzyme activities have been used to describe soil qualities under different agricultural practices (Badalucco *et al*. 2010). Farmyard manure (FYM) and inorganic fertilizers (NPK) have been reported to have both positive and negative effects on the size of MBC and microbial activities (Bohme *et al*. 2005). Soil enzymes allow microbes to access energy and nutrients present in complex substances through nutrient mineralization and humification process. Most of the soil enzyme activities are in direct proportion to native SOC content, reflecting larger microbial communities and stabilization of enzymes on humic substances (Bending *et al*. 2002). Microbial activities particularly population of bacteria, fungi and actinomycetes, MBC and activity of enzymes such as dehydrogenase, urease and phosphatase are the most sensitive indicators of soil health. After crop harvest when the fields are not

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tilled for the next season crop, the soil biological activity reflects the soil's health which is the resultant product of the crop management practices followed over the years. The effect of continuous use of mineral nutrients either singly or in combination is not consistent under all situations. It varies with cropping systems, soil type and agro-climatic situation. Therefore, to get actual effect, location and crop specific studies are necessary. The information will help us in economically managing the soil fertility of a production system for a sustainable crop production. Long-term fertilizer experiments (LTFE) conducted on specific cropping system are thus very useful tool for such study. Our objectives were to determine the effects of five years of continuous application of different nutrients in the form of fertilizers either in pure mineral form or in combination with organic manure on microbial population, MBC and some selected enzyme activities in relation to nutrient availability to a rice-rice system as works on this aspect in this region are very scanty.

Materials and Methods

The long-term fertilizer experiment of All India Coordinated Research Project (AICRP) of ICAR at OUAT, Bhubaneswar, India (20°17′ N, 85°49′ E and 30 m above mean sea level) since 2005-06 was selected for this study. The location of the experimental site is characterized as sub-humid sub-tropical climate with dry season from October to June and wet season from July to September. The soil of the experimental site was sandy loam in texture, acidic (pH 5.8) and udic Ustochrept type. Rice cultivar Swarna (MTU 7029) was grown under flooded condition in both *kharif* and *rabi* season of every year. Five treatments *viz*., 100% N at 80 kg ha^{-1} of N in the form of urea, 100% NP at 80 kg ha^{-1} of N and 40 kg ha^{-1} of P_2O_5 in the form of DAP and urea, 100% NPK at 80:40:40 kg ha^{-1} of $N:P_2O_5:K_2O$ in the form of DAP, urea and MOP; 100% NPK+ FYM at 5 t ha^{-1} and an unfertilized control were evaluated for the study. Nitrogen was applied in three splits *i.e.* 25% as basal, 50% at 18 days after transplanting and 25% at panicle initiation stage. Total P was applied as basal and K was applied 50% as basal and 50% at panicle initiation stage. Rice seedlings were transplanted at a spacing of 20 cm × 10 cm with 2-3 seedlings per hill. Necessary intercultural, water management and plant protection measures were undertaken in general until the crop was matured for harvesting. The experiment was laid out in randomized block design (RBD) and replicated in quadruplicate.

Surface soil samples (0-15 cm depth) were collected during fallow period after harvest of *kharif* rice from each treatment plot. Each soil sample was separated into two parts. One part was air-dried and stored at room temperature for determining soil chemical properties. The other part was passed through a 2-mm sieve, moistened to 60% of their water holding capacity, and immediately stored at 4 °C for the measurement of soil microbial and biochemical properties. Soil pH was determined with a glass electrode pH meter (Jackson 1973). Available N was determined by Kjeldahl method (Subbiah and Asija 1956). Available P was extracted with Olsen reagent (0.5 *M* $NaHCO_3$ at pH 8.5 (Olsen *et al.*1954) at soil extractant ratio of 1:20, shaken for 30 min and quantified by molybdenum-blue colour method using a spectrophotometer. Available K was extracted with neutral normal ammonium acetate (pH 7.0) shaken for 5 min and measured by flame photometer (Jackson 1973). The SOC was determined by dichromate oxidation (Walkley and Black 1934). The MBC was determined by fumigation extraction method (Vance *et al*. 1987). Microbial habitat groups in soil such as bacteria, fungi and actinomycetes were enumerated using nutrient agar, rose Bengal agar and Kenknight's agar as growth medium, respectively, following dilution plating viable count method (Weaver *et al.* 1994). After the required incubation period, the colony forming units (cfu) were counted and expressed as cfu g^{-1} of soil. The acid phosphatase was determined according to Tabatabai (1982) and reported as mg *p*-nitrophenol (PNP) produced g^{-1} dry weight of soil h^{-1}. Urease was determined according to Tabatabai and Bremner (1972) and reported as mg of NH_4^+-N released g^{-1} soil h^{-1}. Dehydrogenase was determined by monitoring the rate of production of triphenyl formazon (Klein *et al.* 1971). All the data were subjected to statistical analysis with software SPSS (Kirkpatrick and Feeney 2005) for significant differences between treatments using analysis of variance (ANOVA) at 5% significance level and correlation between microbial properties and soil available nutrient contents were worked out.

Results and Discussion

Physicochemical properties and available macronutrients

The initial pH of the soil at the start of the experiment was 5.80. The treatment of 100% NPK +FYM showed a pH of 5.88 after five years of continuous cropping which means that the pH has

Table 1. Soil pH and soil organic carbon of rice soil influenced by long-term nutritional managements (Mean of four replications)

Treatment	pH (1:2.5 soil: water suspension)	SOC ($g\ kg^{-1}$)	Available N ($kg\ ha^{-1}$)	Available P ($kg\ ha^{-1}$)	Available K ($kg\ ha^{-1}$)
100% N	5.64^{ab}	4.67^{b}	206.5^{bc}	10.8^{c}	83.1^{b}
100% NP	5.30^{b}	4.15^{b}	193.5^{c}	13.4^{b}	77.4^{b}
100% NPK	5.27^{b}	4.50^{b}	219.0^{ab}	12.0^{bc}	100.3^{a}
100% NPK+FYM	5.86^{a}	5.85^{a}	223.7^{a}	19.6^{a}	110.0^{a}
Control	5.37^{b}	2.35^{c}	133.9^{d}	10.2^{c}	57.8^{c}
Initial	5.80	4.30	187.0	19.4	43.4
SEM (±)	0.11	0.19	8.09	0.55	4.47
LSD (P=0.05)	0.34	0.61	24.9	1.71	13.8

Values followed by the same letter in each column are not significantly different from each other as determined by Duncan's Multiple Range Test ($P<0.05$).

been stable and at par with initial pH. The pH is an intrinsic property of soil which usually does not change easily. Verma *et al.* (2010) in their long-term effects of integrated nutrient management (INM) on soil also found that there was no marked change in soil pH over the years. Over the period of five years of manuring, it revealed that nutrients in balanced and integrated form (100% NPK+FYM) increased the available N content considerably showing an increase of 20 per cent over the initial value (Table 1), while in other treatments, the increase in N availability ranged between 3 and 19 per cent (Table 1). The available N, however, decreased in the control by 28 per cent. In 100% NPK+FYM, the available P showed an increase of 2 per cent over the initial value, but in all other treatments it decreased from 32 to 49 per cent. This shows that FYM along with optimal dose of chemical fertilizer nutrients maintain soil available P. Available K status exhibited a general increase with cropping irrespective of the treatments. The increase in available K over the period ranged between 26 per cent and 157 per cent.

Soil organic carbon

Soil organic carbon (SOC) is centrals to soil fertility. The total SOC content was 4.3 g kg^{-1} at the time of starting the experiment. The total SOC concentration significantly increased with continuous application of organic manure along with inorganic NPK fertilizer over the years, but decreased with only chemical or no fertilization (Control) (Table 1). In our study, it is evident that 100% NPK+FYM application increased SOC by 67 per cent over the initial (4.3 g kg^{-1}). Paustian *et al.* (1992) showed in a 30-year long Swedish field trial that biannual additions of various organic carbon residues like straw, sawdust, green manure and FYM had positive effects on SOC levels. Nevertheless, in unfertilized control, SOC reduced by 26.7 per cent and the decrease in SOC was lesser in inorganic fertilization than the unfertilized control plot which might be due to greater root biomass in the former. Likewise, Ladha *et al.* (2011) showed that long-term use of synthetic fertilizer N lead to a smaller decrease of SOC as compared with no use of synthetic N. This shows that continuous rice cropping without organic matter addition might significantly decrease SOC concentrations, which may then result in soil degradation.

Microbial population

With respect to culturable microbial communities of the rice soil, the bacterial load was highest (Table 2) in 100% NPK+FYM (12.5×10^5 cfu g^{-1} soil), followed by 100% NP (11.3×10^5 cfu g^{-1} soil) and lowest in control (0.16×10^5 cfu g^{-1} soil). Balanced fertilization of N, P and K promoted bacterial biomass growth and improvement of community composition (Zhang and Wang 2005). The fungal and actinomycetes load were also highest in the soil treated with 100% NPK+FYM, followed by NP and lowest in control (Table 2). Both the microbial populations as well as its diversity index increased in presence of FYM and the increase was more than that with inorganic fertilization and control.

Microbial biomass carbon

Distinct differences in MBC content with values lying between 115 to 330.5 mg kg^{-1} soil were observed among the treatments (Fig. 1). The MBC content was the highest in 100% NPK+FYM (330.5 mg kg^{-1} soil) and among only mineral fertilizer added treatments, the 100% N treatment recorded the lowest (135.5 mg kg^{-1} soil) MBC which increased with 100% NP (226

Table 2. Changes in microbial populations (mean of four replications) in long-term fertilized rice soils

Treatment	No. of colony forming units g^{-1} (dry) soil		
	Bacteria ($\times 10^5$ cfu)	Fungi ($\times 10^5$ cfu)	Actinomycet ($\times 10^3$ cfu)
100% N	8.8^c	1.31^c	13.5^c
100% NP	11.3^b	1.51^d	19.5^b
100% NPK	6.4^d	1.03^c	12.0^c
100% NPK+FYM	12.5^a	3.56^a	24.0^a
Control	0.16^e	0.65^e	7.8^d
Initial	2.3	0.98	9.2
SEM (±)	0.21	0.013	0.13
LSD (P=0.05)	0.66	0.041	0.50

Values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test (*P<0.05*).

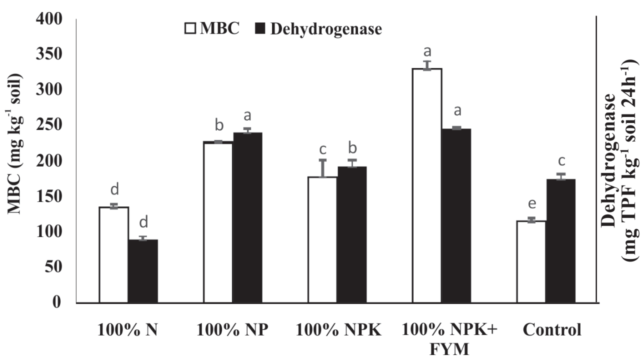

Fig. 1. Impact of long-term nutrient management on changes in microbial biomass carbon (mg kg^{-1} soil) and dehydrogenase activity (mg TPF kg^{-1} soil $24h^{-1}$). Within a parameter, bars followed by different letters are significantly differed at $P<0.05$ as determined by duncan's multiple range test. Each bar represents mean ± SEM (n=4)

mg kg^{-1} soil) and followed by 100% NPK (178 mg kg^{-1} soil). The unfertilized control recorded the lowest MBC among all the treatments. Luo *et al.* (2014) reported that long-term P-deficiency can significantly decrease soil microbial biomass. In our study, organic manure along with inorganic NPK maintained higher available P and recorded greater MBC than the inorganic application and the control. This increase in MBC can be attributed to the incorporation of easily degradable materials, which stimulated the autochthonous microbial activity and to the incorporation of exogenous microorganisms (Tejada *et al*. 2006). It is apparent that MBC is highly correlated with soil organic matter which is in accord with Anderson and Domsch (1980). Based on this long-term experiment, the MBC increased with increased in SOC and MBC was significantly correlated with SOC (r = 0.83**).

Soil enzyme activity

In case of soil enzyme related to C metabolism, dehydrogenase activity was maximum in 100% NPK+FYM plots (246.1 mg TPF kg^{-1} soil $24h^{-1}$) which was followed by 100% NP (240.1 mg TPF kg^{-1} soil $24h^{-1}$) and 100% NPK (192.7 mg TPF kg^{-1} soil $24h^{-1}$) (Fig. 1). The increased dehydrogenase activity might be due to incorporation of organics and owing to increase in microbial activity of soil that also agreed with the results of Sridevi *et al*. (2012). In the treatment of 100% N, dehydrogenase was very low (89.1 mg TPF kg^{-1} soil $24h^{-1}$). The application of N and P fertilizers to rice soils increased the number of both actinomycetes and bacteria but K had no significant effect. The dehydrogenase activity decreased with increased supply of K which might be due to reduced population of fungi. This is supported by Devi (2002). In 100% N, nitrogen alone could not help for more dehydrogenase activity. As P is a major constituent of protein along with N, microbial population could not build-up due to low microbial protein synthesis.

Urease enzyme related to N metabolism was highest in 100% NPK+FYM which was at par with both 100% NPK and 100% NP treatment. Exclusion of both P and K in 100% N treatment recorded lower urease enzyme activity and the unmanured control treatment measured lowest activity (Fig.2). Higher urease activity in 100% NPK+FYM (79.2 mg NH_4^+-N kg^{-1} soil $2h^{-1}$) may be ascribed to the higher populations specifically of anaerobes and actinomycetes which are considered as dominant urease producers (Balamohan *et al.* 2013). Application of inorganic fertilizers and various organic sources had profound influence on the urease activity. The increased rate of N application and various biomaterials added to the soil as well as the root exudates promoted the production of nitrogeneous substance which induced the urease activity (Elayearja and Singravel 2011). It was evident from the result that urease activity was significantly correlated to available N (r = 0.93**).

With respect to hydrolyzing enzymes, the highest acid phosphatase activity was found in 100% NPK+FYM (452 mg PNP kg^{-1} soil h^{-1}) treated plots and the lowest in control (243 mg PNP kg^{-1} soil h^{-1}) (Fig. 2). Phosphatase activity was more in 100% NP treatment than N. Phosphatase activity was significantly and positively related to the available P (r = 0.82**). Phosphatase activity increased with added C suggesting that SOC is a limiting factor for P mineralization (Wang *et al.* 2010). The increase in

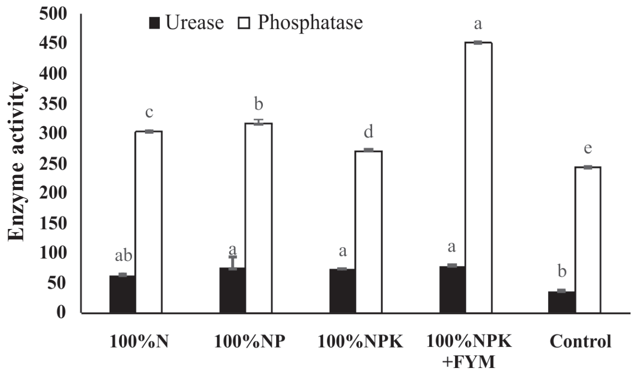

Fig. 2. Impact of long-term nutrient management on changes in urease (mg of NH_4^+-N g^{-1} soil h^{-1}) and phosphatase activity (mg PNP g^{-1} soil h^{-1}). Within a parameter, bars followed by different letters are significantly differed at $P<0.05$ as determined by Duncan's multiple range test. Each bars represents mean ± SEM (n=4)

the soil phosphatase activity with the addition of organics could be attributed to the soil substrate enrichment by combination of mineral fertilizer. The phosphate added through organics and fertilizer improved the phosphatase activity which may be ascribed to the stabilized extracellular fractions of enzyme.

Grain yield and sustainable yield index

Results revealed that the grain yields varied from 1.15 t ha^{-1} in an unmanured control to 3.23 t ha^{-1} in 100% NPK+FYM (Table 3). In 100% NPK+FYM gradual accumulation of organic matter in a light textured acidic soil might be producing a congenial soil environment for higher yield of rice. Among the manured treatments, the lowest yield of 2.04 t ha^{-1} was recorded in 100% N treatment. Application of NP resulted in 17.5 per cent more yield over 100% N treatment which was significantly higher. Application of NPK, however, did not result in any increase in yield. Application of FYM @ 5 t ha^{-1} resulted in 70.5 per cent yield increase. This might be due to improvement in physical, chemical and biological health of the present acidic soil with low organic matter. Similar results have also been recorded in many long-term experiments conducted throughout India and abroad. Yields obtained with either no application or imbalanced nutrition (control, 100% N and 100% NP) was low. The results are in accordance with the results of Stalin *et al.* (2006). Sustainability as measured over 10 cropping seasons with same variety in terms of sustainability yield index (SYI) demonstrated that NPK+FYM treatment was most

Table 3. Effect of long-term manuring on grain yield and sustainable yield index (SYI)

Treatment	Grain yield (t ha^{-1})	SYI
100% N	2.04[b]	0.59
100% NP	2.43[b]	0.65
100% NPK	2.46[b]	0.73
100% NPK + FYM	3.23[a]	0.87
Control	1.15[c]	0.33
SEM (±)	0.13	
LSD(P=0.05)	0.41	

*Values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test ($P<0.05$).

sustainable with SYI of 0.87 followed by NPK treatment (0.73), NP (0.65), N (0.59) and control (0.33).

Statistical analysis of the data showed that grain yield was most significantly correlated with available N (r = 0.93**) and SOC (r = 0.88**).

Conclusions

Our findings clearly indicated that without fertilization acid Inceptisol couldn't support higher grain yield and better soil fertility attributes under rice-rice rotation. Application of balanced mineral fertilizers could maintain higher SYI and the integration of balanced mineral fertilizers with organic inputs further enhance soil fertility status and SYI. Application of only mineral N fertilizer seems to have retarding effect on soil biochemical and biological properties. Moreover, the type of fertilization had impact on soil microbial counts and enzyme activities.

Except for the P-deficient treatment, fertilizer treatments especially organic in combination with inorganic significantly increased MBC, dehydrogenase, urease and phosphatase activity. Integrated nutrient management regime helped in building SOC content and thereby supporting more favourable soil fertility indicators like pH, soil available N, P and K, biochemical and biological properties and overall higher SYI. So, it may be suggested that INM based fertility management strategy for rice-rice rotation in acid Inceptisol could be followed for maintaining higher soil productivity.

Acknowledgements

Authors are grateful to AICRP on LTFE under ICAR for financial and technical support to conduct the experiment.

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Received 06 November 2017; Accepted 13 June 2018