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Soil Enzyme Activities, Microbial Diversity and Available Nutrients Status of an Alfisol under Long Term Fertilization

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A B S T R A C T

The changes in enzyme activities, microbial diversity and nutrients availability in soil under long term fertilization (30 years) with inorganic fertilizers alone or in combination with organics/amendments were investigated in the present study. The experiment consisted of eleven treatments with four replications with the finger millet-maize cropping sequence. Significantly higher biomass C (276.53µg/g) and biomass N (27.20 µg/g) contents were recorded with 100 % NPK+FYM+lime and 100 % NPK+FYM application respectively. Soil enzyme activities (Acid Phosphotase and Dehydrogenase) were higher in these treatments. The general soil microflora was also higher on application of NPK, FYM and lime. The results also envisaged that application of inorganics alone resulted in decreased nutrients status (available NPK) over balanced fertilizer application. Soil acidification was accelerated with application of nitrogenous fertilizers alone (-1.87 unit reduction in soil pH over initial) and the soil pH was maintained in balanced fertilization (6.46). Available nutrients in soil were higher in 100 % NPK+FYM+lime and 100 % NPK+FYM application where the combined application of fertilizers, manure and amendments were undertaken.

Introduction

Studies of microbial biomass C, N and enzyme activities provide information on the biochemical processes occurring in the soil and there is growing evidence that soil biological parameters may have a potential as early and sensitive indicators of soil ecological stress and restoration (Dick and Tabatabai, 1992). Soil microbial diversity is one of the most important microbial parameters in soil. It has been demonstrated that soil microbial diversity is affected by anthropogenic disturbance (Fox and MacDonald, 2003). Long-term experiments point to a complex of direct and indirect changes in physicochemical and biological soil properties affected by the application of organic and mineral fertilizers or no fertilizers at all. Fertilization affects soil properties essential for its agricultural quality and ecological balance: the content and transformations of organic carbon (Kubat *et al*., 2006), acidification and soil reaction (Debreczeni and Kismanyoky, 2005) nutrients contents as well as their availability to plants (Madaras and Lipavsky, 2009).

Microbial community plays a vital role in regulating processes such as decomposition of

organic matter and nutrient cycling in soil at the ecosystem level (Zeller *et al*., 2001). The importance of the size of microbial biomass is emphasised by the fact that this is the eye of the needle through which all organic material that enters the soil must pass. Besides the size of microbial biomass, its functional and structural diversity has relevance as well. Functional diversity (e.g., microbial activity) is significant, because 80–90% of the processes in soil are reactions mediated by microorganisms (Nannipieri and Badalucco, 2003 and Livia Bohme *et al*., 2005). Among agricultural practices, ploughing, manuring and fertilization and crop rotation have beneficial, harmful or neutral effects on the trinity formed by plants, soil organisms (microbes and fauna) and soil (Bowen and Rovira, 1991 and Mandal *et al*., 2007).

Application of either alone or dual fertilizers resulted in soil nutrients imbalance, soil acididy and poor crop performance. These changes, in the long-term, are believed to have significant influences on the quality and productive capacity of the soil (Acton and Gregorich, 1995). Effects of management practices on soil quality and productivity are best evaluated using long-term experiments. A long term fertilizer experiment at Bangalore, India was started in 1986-87 with finger millet – maize cropping sequence on a typic kandicustalfs. The objective of the study was to study the soil enzyme activities, microbial diversity and available nutrients status in soil under long term fertilization.

Materials and Methods

Experimental details

The experimental site is gio-positioned at an altitude of 930 meters above MSL, latitude of 13° north, longitude of 77″3′ east. The annual rainfall occurs from April to November with an average rain fall of 920.4 mm. There are eleven fixed treatments established in permanently laid out plots in randomized block design with four replications on finger millet – maize cropping sequence. Neither the treatments nor the management practices in respect of fertilizers doses, irrigation and plant protection measures have changed over the years. The treatments details are as under.

T_1: 50% NPK
T_2:100%NPK
T_3:150%NPK
T_4:100%NPK+HW
T_5:100%NPK+Lime
T_6:100%NP
T_7:100%N
T_8:100%NPK+FYM
T_9:100%NPK(S-free)
T_{10}:100%NPK+FYM+lime
T_{11}: Control

Lime, as per lime requirement test is applied only when found necessary, during the kharif season. Well decomposed Farmyard manure (FYM) at the rate of 15 t ha^{-1} on dry weight basis is incorporated into the soil 10-15 days before sowing of the *kharif* crop. Half the dose of the nitrogen, full dose of P and K applied as basal and remaining half of nitrogen dose is applied after 25 to 30 days of sowing / transplanting of crops as top dress. Diammonium phosphate (DAP) is used as a source of P and N along with urea and muriate of potash (MoP) in 100% NPK (-S). For all the treatments (except 100% NPK -S), urea, single super phosphate are used assources of NPK fertilizers. Neither any chemical fertilizer nor any organic manure is used in absolute Control (No NPK) treatment.

Microbial biomass carbon and nitrogen

Microbial biomass was estimated using the $CHCl_3$ fumigation-extraction method (Vance *et al*., 1987). Samples of moist soil (10 g) were used, and K_2SO_4-extractable C was

determined using dichromate digestion. Microbial biomass N was calculated as a difference in N content in fumigated and non-fumigated sample (E_N) using k_{EN} coefficient (microbial biomass N = E_N:k_{EN}). The value of k_{EN} = 0.54 was used to calculate microbial biomass N (Jenkinson, 1988).

Dehydrogenase activity

Soil samples (3 g) were mixed with 0.04 g $CaCO_3$, 1 ml of 3 % aqueous triphenyl-tetrazolium chloride (TTC) solution and 2.5 ml of distilled water in test tubes. The tubes were sealed, shaken and incubated at 37°C for 24 hr. TTC-formazan was extracted from the soil suspension with CH_3OH, filtered, and made up to 50 ml with additional CH_3OH. The absorbance at 485 nm of the extracts was measured by spectrophotometer (Shimadzu UV-1800) using CH_3OH as a blank by following the method as outlined by Casida *et al*., (1964).

Acid phosphatase activity

Acid phosphatase activity was assayed using 1 g of soil (wet equivalent), 4 ml of 0.1 M modified universal buffer (pH 6.5), and 1ml of 25 m M p-nitrophenyl phosphate. After incubation for 1 hr at 37±1°C, the enzyme reaction was stopped by adding 4 ml of 0.5 M NaOH and 1 ml of 0.5 M $CaCl_2$ to prevent dispersion of humic substances. After centrifugation at 4000 rpm for 10min, the absorbance was measured in the supernatant at 400 nm; enzyme activity was expressed as µg/PNP/g/24 hr.

Microbial population

Ten gram of pooled soil was mixed in 90 ml sterilized blank to give 10^{-1} dilution subsequent dilutions to 10^{-6} were made by transferring serially 1ml of the dilution to 9 ml of sterilized blank. The populations of bacteria, fungi, and actinomycetes were estimated by transferring 1 ml of 10^{-6} and 10^{-3} and 10^{-4} dilutions respectively to a sterile petridish and approximately 20ml of media *viz*., soil extract agar for soil bacteria, Martin's rose Bengal agar for fungi and Kuster's agar for actinomycetes respectively was poured into plates the plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidification of media, plates were kept for incubation in an inverted position at 30°C for a week time and emerged colonies were counted (Tate, 1995).

Soil analysis

Soil samples were collected from 0- to 15-cm soil depth after the harvest of maize during 2015 the samples was air dried, ground and passed through 2-mm sieve for further analysis. The pH of the soil was determined in 1:2.5 soil: water suspension using pH meter (Jackson, 1967). The electrical conductivity of the soil samples was measured in the supernatant liquid of 1:2.5 suspension using a conductivity bridge (Jackson, 1973). Soil organic C concentration was estimated from soil samples through wet oxidation method (Walkley and Black, 1934). The available N (alkaline permanganate method, Subbiah and Asija, 1956); Available P was extracted with NH_4F-HCl solution (Bray and Kurtz, 1945), available potassium was extracted from 1N NH_4OAC-K (Hanway and Heidel, 1952). The soil is typic kandicustalfs with sandy clay loam texture. Initially the soil reaction was acidic (6.17), low in organic carbon content (0.46 %) and available NPK contents of the soil are 256.7 kg ha^{-1}, 34.30 kg ha^{-1} and 123.10 kg ha^{-1} respectively.

Statistical analysis

In order to compare the treatments, the data was pooled over the years and an analysis of

variance (ANOVA) was performed following standard procedures for randomized block design (Gomez and Gomez, 1984). The F-test was used to test significant differences between treatment means. The significant differences between treatments were compared with the critical difference (C.D.) at 5 % level of probability.

Results and Discussion

Enzyme activities and microbial diversity

Significantly higher biomass C was recorded on application of 100 % NPK+FYM+lime (276.53µg/g) compared to all other treatments. Among the treatments there was no significant difference with respect to microbial biomass N (Table 1). Phosphatase activity was significantly higher in 100 % NPK+FYM+lime (118.77 µg/PNP/g/24hr) compared to all other treatments. Dehydrogenase activity recorded a significantly higher activity in 100 % NPK+FYM (65.80 µg/TPF/g/24hr) over all the other treatments. It was noticed that the treatments with combined application of FYM and chemical fertilizers recorded higher biomass and greater enzyme activities compared the inorganic fertilizers alone. The greater activities of phosphatase, in the FYM treated soils may be due to enhanced microbial activity and diversity of phosphate solubilizing bacteria due to manure input over the years. The dehydrogenase activity in this study could not be related to soil organic C or to microbial biomass C. Dehydrogenase activity, as a measure of soil microbial activity, is strongly influenced by the presence of nitrate, which serves as an alternative electron acceptor resulting in low activities (Sneh Goyal *et al*., 1999). Dehydrogenase was highly sensitive to the inhibitory effects associated with large fertilizer additions. The effects of fertilization on dehydrogenase activity may be direct, related for example to changes in the availability of nutrients or heavy metals present in the fertilizers as contaminants (Simek *et al*., 1999).

Among the general microflora significantly higher bacterial population (Fig. 1) was observed in 100 % NPK+FYM+lime application (31.33 cfu g dry wt. soil^{-1}), which was superior over rest of the treatments. The fingal population also deferred significantly wherein application of 100 % NPK+FYM+lime (17.67 cfu g dry wt. soil^{-1}) and 100 % NPK+FYM (17.67 cfu g dry wt. soil^{-1}) recorded the higher population of fungi. Actinomycetes population was significantly higher in 100 % NPK+FYM+lime application (7 cfu g dry wt. soil^{-1}), 100 % NPK+FYM (7 cfu g dry wt. soil^{-1}) and 150 % NPK (7 cfu g dry wt. soil^{-1}) which were significantly superior over all the other treatments. Lower population of bacterial, fungal and actinomycetes (15.33, 5.33 and 2 cfu g dry wt. soil^{-1}) were recorded in absolute control. Use of FYM alone or in combination with chemical fertilizers led to higher numbers of microbes and enhanced microbial respiration than use of chemical fertilizers alone. Farm manure is rich in organic matter and an important source of nutrients for plants and microorganism in soil, its incorporation into soil promotes microbiological activities and improves chemical fertilizer use efficiency. Bacteria were more numerous (1×10^5 cfu (colonies forming units) g dry wt. soil^{-1}) than fungi (1×10^3cfu g. dry wt. soil^{-1}) which may lead to more soil organic matter (SOM) mineralization and less SOM retention in this cropping system (Fig. 1). It is evident from the study that in treatments receiving farm yard manure microbial population were higher compared to the no FYM applied plots and this may be attributed to more availability of carbon (Belay *et al*., 2002). The results indicates that due to acidification as accelerated by the chemical fertilizers

(especially urea), the soil reaction was reduced (Fig. 2) when compared to the initial pH (6.17) except in 100 % NPK+FYM+lime (6.46) and control (6.40). The magnitude of reduction was slightly higher where only N was applied (4.30) followed by 100 % NP (4.73) this was mainly due to soil acidification caused by the synthetic nitrogen fertilizer.

Table.1 Effect of long term fertilization and cropping on enzyme activities

Treatments	Microbial Biomass C µg/g	Microbial Biomass N µg/g	Acid Phosphotase µg/PNP/g/24hr	Dehydrogenase µg/TPF/g/24hr
T_1:50% NPK	229.36	24.0	88.36	56.20
T_2:100%NPK	241.06	25.1	92.43	62.00
T_3:150%NPK	264.20	26.7	94.59	64.40
T_4:100%NPK+HW	238.46	26.8	88.56	52.00
T_5:100%NPK+Lime	236.40	25.0	87.79	59.80
T_6:100%NP	204.96	23.6	88.11	42.20
T_7:100%N	206.97	23.9	85.21	43.60
T_8:100%NPK+FYM	262.50	27.2	102.37	65.80
T_9:100%NPK(S-free)	237.06	24.6	88.57	33.80
T_{10}:100%NPK+FYM+Lime	276.53	26.1	118.77	60.20
T_{11}:Control	216.36	23.4	81.73	47.00
CD @ 5%	**9.02**	**4.25**	**12.99**	**4.65**

Table.2 Available nutrients status in soil after 28th cycle of finger millet- maize cropping sequence

Treatments	Avail.N	Avail. P_2O_5	Avail. K_2O	Exch.Ca	Exch.Mg	Avail.S
	(kg/ha)			(c mol p^+/kg)		(kg/ha)
50% NPK	179.69	61.02	142.11	5.18	3.03	28.72
100%NPK	206.65	83.06	170.35	4.25	2.50	28.24
150%NPK	262.80	109.15	229.16	4.43	2.93	28.13
100%NPK+HW	215.20	79.54	180.84	5.00	2.65	27.21
100%NPK+Lime	226.27	82.23	185.48	5.85	2.90	29.28
100%NP	214.18	79.07	80.00	4.08	2.68	28.54
100%N	223.29	42.48	72.84	3.85	2.20	28.54
100%NPK+FYM	284.35	88.81	200.57	4.75	3.08	29.33
100%NPK(S-free)	217.20	85.33	179.42	4.43	2.55	28.89
100%NPK+FYM+lime	289.37	94.54	213.45	6.50	3.50	29.81
Control	172.01	38.89	88.54	5.00	3.00	29.57
SEm±	5.57	2.90	7.37	0.26	0.20	0.74
CD @5%	16.44	8.55	21.73	0.77	0.60	2.19
Initial	**257.0**	**34.3**	**123.1**	**3.25**	**1.55**	**9.06**

Fig.1 Effect of long term fertilization and cropping on microbial diversity

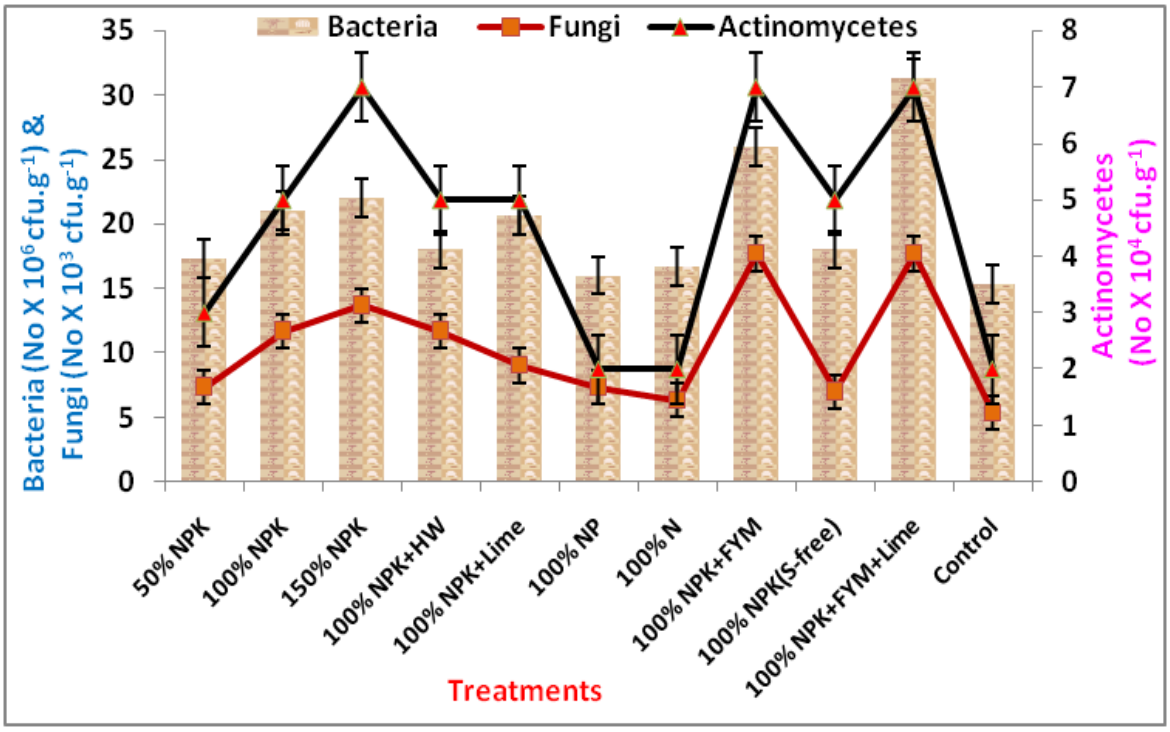
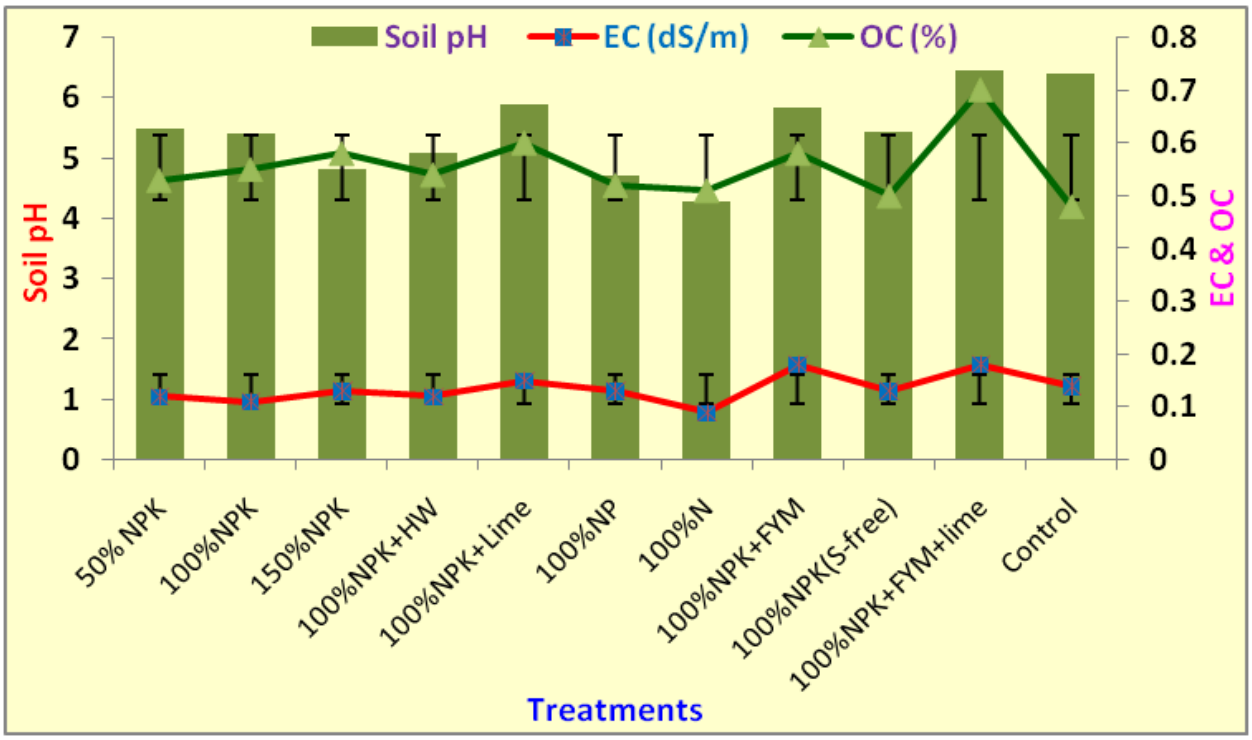


Fig.2 Effect of long term fertilization on soil pH, electrical conductivity and organic carbon content



The organic carbon content in all the treatments was slightly increased over its initial status (0.46 %) due to C addition through the roots and crop residues, higher humification rate constant, and lower decay rate (Kundu *et al*., 2002 and Enke Liu *et al*., 2010). Significantly higher organic carbon content was recorded in 100 % NPK+FYM+lime (0.70) followed by 100 % NPK+lime (0.60 %). Higher soil organic carbon content was noted on combined application of FYM and mineral fertilizers (Bhattacharyya *et al*., 2010). Lower OC contents were observed in control (0.48 %) and in 100 % N alone (0.50 %).

Among the major nutrients status in soil, available nitrogen content of soil has decreased in all the treatments except in 100 % NPK+FYM+lime 100 % NPK+FYM and 150 % NPK, the magnitude of nitrogen loss was higher in absolute control where there was no application of fertilizers (Table 2). This indicated that the loss of nitrogen is higher over its application due to crop removal and other losses. Application of 100% NPK + FYM and super optimal dose (150% NPK) recorded a significant build-up of available P followed by all other treatments. The long-term continuous inorganic fertilizer application, had a significant contribution to soil P availability and its build up in soil due to soil fixation (Wang *et al*., 2010). Maximum potassium buildup was recorded on application of 150 % NPK (106.06 kg ha^{-1}) followed by 100 % NPK+FYM+lime and other treatments, the available potassium content in soil was depleted in treatments where K was not applied (T_6, T_7 and T_{11}).The depletion of major nutrients status in soil was due to higher crop removal, imbalanced nutrition or no application of fertilizers. Regular application of lime and FYM resulted in build of phosphorus and potassium. The present results corroborated the findings of Jaskulska *et al*., (2014). The secondary nutrients statues in soil found to increase in all the treatments over the initial values, however application of balanced fertilizers resulted in higher buildup in soil compared to absolute control and inorganics alone. The increase in these nutrients contents is due to application of chemical fertilizers, farm yard manure and lime which contained appreciable amounts of these elements.

In conclusion, balanced nutrition (100 % NPK+FYM+lime) ensured greater microbial activities and higher microbial population suggesting their vital role as a part of sustainable agriculture. Application of balanced fertilizers along with organic manure and amendments could result in maintaining and sustaining the soil fertility and productivity over the years. Application of chemical fertilizers alone resulted in soil acidification up to 1.87 unit reduction over the original value wherein application of 100 % NPK+FYM+lime maintained the soil pH (6.46) compared to all the other treatments. Application of farm manure at 10 t ha^{-1} along with recommended dose of fertilizers and lime found promising in term of sustaining crop and soil productivity. There was buildup of phosphorus and potassium in soil over the initial status.

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