Original Research Article

Nine years of integrated nutrient management practices on soil microbial activities in a cereal-based cropping system

ABSTRACT

The present work is a follow-up study to identify the effects of nine years long-term integrated nutrient management practices on soil microbial activities in a cereal-based cropping system which was initiated in 2010. The microbial activities like microbial population and enzyme activities were estimated at three different soil depths; 0-15cm, 15-30cm and 30-45cm, in response to different treatment regimes of inorganic fertilizers, organic manures, biofertilizers, and lime. The results identified a decreasing trend in the microbial population and enzyme activity with increased soil depth. Of all the treatment regimes, the maximum enhancements of dehydrogenase and urease activities were observed when there was a combined application of lime with inorganics and organic manure. Additionally, this treatment also increased the bacterial population while decreasing the fungal and actinomycetes population in the soil. The increased soil microbial activities in this treatment can be attributed to the role of organic manures.

Keywords: Acidic Inceptisols, cereal based cropping system, integrated nutrient management, microbial population, enzyme activity

1. INTRODUCTION

One-third of the earth's surface area and about half of the world's potentially cropped land is acidic [1]. In India, soil acidity affects around one-third of the cultivated lands. Maximizing crop yields with good soil health and proper environmental and ecological balance is possible only with the balanced use of mineral fertilizers and organic sources of nutrients such as organic manures like farmyard manure (FYM), compost, green manures, and biofertilizers [2–4]. Application of organic manures like vermicompost having C:N ratio below 15 is suitable for crop production [5].Sustainable crop production has become a great challenge now a days, particularly in problem soils like acid soil. Long-term experiments have shown that neither organic sources nor mineral fertilizers alone can achievesustainability in crop production. The integrated use of organic and inorganic fertilizers is more effective in maintaining higher productivity and stability [2,3,6,7].The use of imbalanced and inadequate fertilizers and restricted use of organic manures have made the soils not only deficient in nutrients but also deteriorate soil health [8] and decreased the yield of the crop.

To supply a recommended dose of nutrients, large quantities of organic materialsare needed, and also a slow release of plant nutrients upon decomposition from organic material deprives crop growth [9–11]. Microorganisms play a very crucial role in soil fertility. They

also play an important role in the degradation of organic matter and also in the detoxification of toxic wastes and pollutants. Microbial activities particularly the population of bacteria, fungi, and actinomycetes, microbial biomass carbon, and activity of enzymes such as dehydrogenase, urease, and phosphatase are the most sensitive indicators of soil health [12]. Interest in soil enzyme activity has increased recently since their activities are believed to reflect the potential capacity of soil to perform nutrient transformations. By considering the above facts, this experiment was carried out to evaluate the change in biological properties in acid *Inseptisols* in different depths.

2. MATERIALS AND METHODS

The present long-term integrated nutrient management (INM) field experiment on cereal-vegetable-pulse cropping system was performed on the farmland of "AINP on Soil Biodiversity - Biofertilizers" in the College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar situated at 20.26°N latitude, 85.81°E longitude and 25.9 m above mean sea level. The experimental area falls under a subhumid tropical climate. The mean annual rainfall was 1577 mm, and the mean maximum and minimum temperatures were 33.2 and 21.4°C, respectively.

The soils of the site belong to *Inceptisols* order with acidic soil reaction. The experiment was initiated in the year 2010, during 2018-19 the cropping cycle was sweetcorn, knolkhol, and blackgram. The experiment was laid out in a randomized block design (RBD) having three replications with treatments consisting of; T1 (control), T2 (NPK), T3 (NPK+FYM), T4 (NPK+VC), T5 (NPK+FYM+BF), T6 (NPK+VC+BF), T7 (NPK+FYM+Lime+BF), T8 (NPK+VC+Lime+BF), T9 (1/2 NPK+BF) and T10 (uncultivated Fallow). The NPK dose was given to the crop as per the soil test-based dose viz; 150:30:60 for sweetcorn, 150:40:60 for knolkhol, and 20:20:30 for blackgram in the form of $N:P_2O_5:K_2O$ kg ha^{-1}. Lime was applied @ 0.1 LR to sweetcorn and @0.2 LR to knolkhol and blackgram crop. Standard methods were adopted for the analysis of soil and organic inputs [13,14]. Organic sources applied were farm yard manure (FYM) @ 5t ha^{-1} and vermicompost (VC) @ 2.5 t $ha^{-1}$to each crop. Biofertilizers (BF) like *Rhizobium* to Blackgram and *Azotobacter, Azospirillum* and PSB (@1:1:1) to Knolkhol and Sweetcorn.The crop residues were incorporated into the soil after harvesting the economic yield potion of each crop.Representative soil samples were collected at the end of the ninth cropping cycle after harvesting blackgram in June 2019.

The collected fresh soil samples were stored immediately in a refrigerator at 4°C for microbial parameter analysis. Microbial populations in the soil such as bacteria, fungi, and actinomycetes were enumerated using nutrient agar, rose Bengal agar, and Kenknight's agar as a growth medium, respectively, following the dilution plating viable count method [15]. After the required incubation period, the colony forming units (cfu) were counted and expressed as cfu g^{-1} of soil.Urease activity was determined according to [16] and reported as mg of NH_4^+ -N released g^{-1} soil h^{-1}. Dehydrogenase activity was determined by monitoring the rate of production of triphenyl formazan [17,18]. All the data were subjected to statistical analysis with software SPSS [19] for significant differences between treatments using analysis of variance (ANOVA) at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1 INFLUENCE OF NINE-YEAR LONG-TERM INM PRACTICES ON MICROBIAL POPULATION

3.1.1 INFLUENCE ON BACTERIAL POPULATION

The data relating to bacterial populations in different depths of soil has been presented in Table 1. The heterotrophic bacteria population in 0-15 cm of soil was more than 15-30cm and 30-45 cm depth soil. The heterotrophic bacteria population on the surface soil (0-15cm) was highest (63) in the integrated package where lime was applied with a soil test dose of NPK, vermicompost and biofertilizers followed by a soil test dose of NPK, farm yard manure and biofertilizers (60), NPK + VC+BF (55), NPK + FYM+ BF (52), NPK +VC (37), ½ NPK + BF (37), NPK +FYM (35), Fallow (32), Control (29) and lowest was in completely inorganic package NPK (25). There was no significant difference (p=0.05) between vermicompost and farm yard manure applied packages. In 15-30 cm depth, the bacterial population was more than 30-45 cm soil but less than 0-15 cm depth soil. The heterotrophic bacteria population in 15-30 cm depth varied between 14 and 50. The highest was in T8 (50) followed by T7 (46), T6 (42), T5 (38), T4 (29), T9 and T3 (25), T10 (21), T1(16), and the lowest was in T2 (14).

The bacterial population in the subsurface layer (15-30 cm) were significantly different among themselves (p=0.05) except for control and NPK packages where these two packages were statistically at par (p=0.05).The bacterial population in the deeper layer (30-45 cm) was ranged between 10-14 x 10^5cfu g^{-1} soil and it was nonsignificant among the packages. In the vermicompost applied packages, the bacterial population were more than in farm yard manure applied packages due to more readily available nutrient for microbes. The addition of biofertilizer with organics enhances the bacterial population, and ameliorants like lime improved the bacterial population. The liming neutralizes the soil acidity, enhances the nutrient availability to the plant, and thus provides a congenial environment to the microbes [20]. The biofertilizers containing rhizobium secrets exopolysaccharide, phytohormone like indole acetic acid [21], and other metabolites which enhance the microbial population in the rhizosphere [22]. Integrated nutrient management with soil amelioration enhances the biological properties [12]. The application of only inorganic nutrients depletes the soil reaction (pH) [23] which resulted in the depletion of the bacterial population.

Table 1: Influence of nine years of INM practice on heterotrophic bacterial population (x 10^5cfu g^{-1} soil)

	Depth of Soil (cm)		
Treatments	**0-15**	**15-30**	**30-45**
T1: Control	29	16	11
T2: NPK	25	14	10
T3: NPK+FYM	35	25	11
T4: NPK+VC	37	29	12
T5: NPK+FYM+BF	52	38	12
T6: NPK+VC+BF	55	42	13
T7: NPK+FYM+Lime+BF	60	46	14
T8: NPK+VC+Lime+BF	63	50	14
T9: 1/2 NPK+BF	37	25	11
T10: Fallow	32	21	10
LSD (5%)	7.4	4.5	NS

3.1.2 INFLUENCE ON FUNGAL POPULATION

The data related to the influence of long-term manurial practice on soil fungal populations in different depths of soil has been presented in Table 2. The surface soil (0-15 cm) had a maximum fungal population than sub-surface (15-30 cm) and deeper soil (30-45 cm). The fungal population at 0-15 cm, 15-30 and 30-45 cm depth soil was varied between 22 x10^4

cfug^{-1} soil and 35 x10^4 cfu g^{-1} soil; 12 x10^4 cfu g^{-1} soil and 22 x10^4 cfu g^{-1} soil; and 9 x10^4 cfu g^{-1} soil and 12 x10^4 cfu g^{-1} soil, respectively. In 0-15 cm layer of soil, the highest was observed in NPK+FYM+BF (35 x10^4 cfu g^{-1} soil) followed by NPK+VC+BF (33 x10^4 cfu g^{-1} soil), NKP+FYM (30 x10^4 cfu g^{-1} soil), NPK + VC (30 x10^4 cfu g^{-1} soil), NPK (27 x10^4 cfu g^{-1} soil), NPK + FYM +Lime + BF (25 x10^4 cfu g^{-1} soil), NPK + VC+ Lime +BF and control (22 x10^4 cfu g^{-1} soil), 1/2 NPK + BF (20 x10^4 cfu g^{-1} soil), and lowest was in fallow (18 x10^4 cfu g^{-1} soil). The application of lime reduced the fungal population, it was due to the reduction of soil acidity as fungus can grow in a wide range of pH [24,25]. The fungal population was more in FYM applied packages than VC applied packages. In 15- 30 cm soil the fungal population was highest (22 x 10^{4}cfu g^{-1} soil) in T5 followed by T3 (20 x 10^{4}cfu g^{-1} soil), T6 and T4 (19 x 10^{4}cfu g^{-1} soil), T9 (16 x 10^{4}cfu g^{-1} soil), T7 and T2 (15 x 10^{4}cfu g^{-1} soil), T10 (13 x 10^{4}cfu g^{-1} soil), T1 (12 x 10^{4}cfu g^{-1} soil) and the lowest was in T8 (11 x 10^{4}cfu g^{-1} soil). In 30-45 cm soil the fungal population was nonsignificant (p=0.05) with each package.

Table 2: Influence of nine years of INM practice on fungal population (x 10^4 cfu g^{-1} soil)

	Depth of Soil (cm)		
Treatments	**0-15**	**15-30**	**30-45**
T1: Control	22	12	9
T2: NPK	27	15	10
T3: NPK+FYM	32	20	12
T4: NPK+VC	30	19	10
T5: NPK+FYM+BF	35	22	12
T6: NPK+VC+BF	33	19	10
T7: NPK+FYM+Lime+BF	25	15	10
T8: NPK+VC+Lime+BF	22	11	9
T9: 1/2 NPK+BF	20	16	11
T10: Fallow	18	13	10
LSD (5%)	4.3	2.3	NS

3.1.3 INFLUENCE ON ACTINOMYCETES POPULATION

The data related to the actinomycetes population of soil in different soil depth has been presented in Table 3. The actinomycetes population in 0-15 cm soil layer ranged from 10 x 10^{4}cfu g^{-1}soil to 22 x 10^{4}cfu g^{-1}soil. The highest was in the package where soil test-based fertilizer was applied with farm yard manure and biofertilizers (T5) followed by package where soil test-based fertilizer was applied with vermicompost and biofertilizers (T6), NPK + FYM (T3), NPK + VC (T4) and ½ NPK + BF (T9), fallow (T10), NPK+ FYM +Lime+ BF (T7) and control (T1), NPK+ VC+ Lime+ BF (T8) and lowest was in NPK (T2). In 15-30 cm depth of soil the actinomycetes population was varied between 6 x 10^{4}cfu g^{-1}soil and 15 x 10^{4}cfu g^{-1}soil. The actinomycetes population in 30-45 cm depth of soil was ranged from 4 x 10^{4}cfu g^{-1}soil to 7 x 10^{4}cfu g^{-1}soil. The actinomycetes population was nonsignificant (p=0.05) among the packages. The data revels that the actinomycetes population was influenced positively by FYM application and negatively with liming of acid soil. Similar finding was reported by [26] in rhizosphere of red gram and [12] in rice-rice ecosystem.

Table 3: Influence of nine years of INM practice on actinomycetes (x 10^4 cfu g^{-1} soil) population

	Depth of Soil (cm)		
Treatments	**0-15**	**15-30**	**30-45**
T1: Control	12	8	5
T2: NPK	10	6	4
T3: NPK+FYM	18	12	5
T4: NPK+VC	15	10	5
T5: NPK+FYM+BF	22	15	7
T6: NPK+VC+BF	19	12	6
T7: NPK+FYM+Lime+BF	12	7	6
T8: NPK+VC+Lime+BF	10	6	5
T9: 1/2 NPK+BF	15	10	6
T10: Fallow	13	8	5
LSD (5%)	2.3	1.6	NS

3.2 INFLUENCE OF NINE YEARS OF INM PRACTICES ON SOIL ENZYME ACTIVITIES

3.2.1 INFLUENCE ON SOIL DEHYDROGENASE ACTIVITY

The dehydrogenase activity of the soil was presented in Fig 1. The activity of dehydrogenase enzyme in 0-15 cm soil depth was varied between 20 µg TPF g^{-1}dw to 69 µg TPF g^{-1}dw. The highest was estimated in the integrated package where soil test dose of NPK along with vermicompost, lime and biofertilizer was applied (69 µg TPF g^{-1}dw) followed by the package where soil test dose of NPK along with farmyard manure, lime and biofertilizer was applied (60 µg TPF g^{-1}dw), soil test dose of NPK with vermicompost and biofertilizer (54 µg TPF g^{-1}dw), soil test dose of NPK with farmyard manure and biofertilizer (48 µg TPF g^{-1}dw), soil test dose of NPK along with vermicompost was applied (40 µg TPF g^{-1}dw), ½ of soil test dose of NPK with biofertilizer (35 µg TPF g^{-1}dw), soil test dose of NPK with farmyard manure (33µg TPF g^{-1}dw), uncultivated fallow (28 µg TPF g^{-1}dw), control (23 µg TPF g^{-1}dw)and lowest was in soil test dose of NPK (20 µg TPF g^{-1}dw).

Fig 1. Influence of nine years of INM practice on soil dehydrogenase activity (µg TPF g^{-1}dw)

In 15-30 cm depth the dehydrogenase activity was highest in T8 (55 µg TPF g^{-1}dw) followed by TT7 (52 µg TPF g^{-1}dw), T6 (46 µg TPF g^{-1}dw), T5 (42 µg TPF g^{-1}dw), T4 (33 µg TPF g^{-1}dw), T9 (30 µg TPF g^{-1}dw), T3 (27 µg TPF g^{-1}dw), T10 (22 µg TPF g^{-1}dw), T2 (19 µg TPF g^{-1}dw), and lowest was in control (17 µg TPF g^{-1}dw). The dehydrogenase activity of soil in 30-45 cm depth was varied between 12 µg TPF g^{-1}dw and 17 µg TPF g^{-1}dw. The dehydrogenase activity was nonsignificant among the treatments. The dehydrogenase activity was decreased with increase in depth of soil. Liming of acid soil increased the dehydrogenase activity. The dehydrogenase activity was more in organic applied packages than inorganic packages. The application of organics increased the dehydrogenase activity due to degradation of added materials which provides intra and extracellular enzymes and increase microbial activity in the soil [27,28]. The dehydrogenase activity of organic management soil was more than conventional management [29,30].Liming of acid soil enhanced the dehydrogenase activity of soil by creating a congenial environment for microbial growth [31] The finding of this experiment was also agreed with the findings of [12,32,33].

3.2.2 INFLUENCE ON SOIL UREASE ACTIVITY

The urease activity of the soil in different depth has been presented in Fig 2. The urease activity in the 0-15 cm soil depth was varied between 5 µg NH_4^+ g^{-1} h^{-1} and 15 µg NH_4^+ g^{-1} h^{-1}. The highest (15 µg NH_4^+ g^{-1} h^{-1}) was in lime applied treatments irrespective of the types of organic fertilizers followed by integrated management package without lime and without biofertilizer package. The urease activity of only inorganic package (5 µg NH_4^+ g^{-1} h^{-1}) was lower than control (7 µg NH_4^+ g^{-1} h^{-1}). The urease activity of fallow (9 µg NH_4^+ g^{-1} h^{-1}) was more than control and only inorganic package but these were statistically at par (p=0.05). In 15-30 cm depth the urease activity was ranged from 4 µg NH_4^+ g^{-1} h^{-1} to 13 µg NH_4^+ g^{-1} h^{-1}. The urease activity in 30-45 cm depth was varied between 2.3 µg NH_4^+ g^{-1} h^{-1} and 5.1 µg NH_4^+ g^{-1} h^{-1} and all the packages were statistically at par (p=0.05). Similar findings have been reported by [12,34].

Fig 2. Influence of nine years of INM practice on soil urease activity (µg NH_4^+ g^{-1} h^{-1})

Our results establish that in this studied sustainable ecosystem it depends on the nutrient flows through the trophic levels, which are mainly mediated by microorganisms [35]. Due to environmental degradation and poor agricultural practices, the intrinsic biodiversity of soils has been subject to numerous variations, which has led to changes in the functioning of the native microbial communities of these soils [36]. As a consequence, the outcome of these modifications, in terms of the ability of ecosystems to maintain ecosystem functions and services, is of fundamental importance. In this context, it is a challenge to understand and predict the mechanisms that govern the actions of soil microbial diversity and the relationship between that biodiversity and other processes that occur in it [37].

The functions performed by microorganisms are essential for crop growth [38]. These functions include the decomposition of OM in all its fractions, the recycling of plant material, the mobilization and immobilization of minerals and pollutants, the improvement in soil aeration, the inhibition of pathogens, the increase in resistance in plants, the physical structuring of the soil and the increase in plant nutrition promoting its growth, among others [39]. For this reason, the processes in which microorganisms participate are strongly influenced by the agricultural management under which they are found [40].

4. CONCLUSION

From this experiment, it can be concluded that the microbial activities *viz*; microbial population, enzyme activities in soil decreases with increase in soil depth under INM package of practices over nine years of cereal-based cropping. The liming of acid soil under this cropping system enhanced the heterotrophic bacteria population in soil and enzyme activities like dehydrogenase and urease whereas the fungal and actinomycetes population in soil depleted. Only inorganic application depletes the microbial activity in terms of bacterial populations and both the enzyme activities. The addition of organics like, farm yard manure and vermicompost to soil increases over all microbial activity.

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