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Study on the Effect of Long Term Manurial Experiment on Sustain Soil Health and Crop Yields

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ABSTRACT

Field experiment is conducted to study the effect of soil health on continuous manuring on crop yield , organic carbon status and soil microbial activity of 112 years of Permanent Manurial Experiment. We examined the positive impact of organic manure which applied along with mineral fertilizers compared to the inorganic fertilizers alone. Over the years result revealed that the increase in organic carbon status in the treatment receiving NPK + FYM (INM) which helps to improve soil microbial activity and crop yield and which play great role in mitigating climate change.

Keywords Permanent manurial experiment, Soil organic carbon, Maize yield, Microbial activities.

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INTRODUCTION

Soil organic carbon contents do not produce significant changes in short term experiments; therefore management impacts cannot be completely studied (Paul *et al*. 2001). This warrants long term experiments, since it will provide an excellent opportunity to determine the effects of organic amendments on SOC dynamics. Several long term fertilizer studies have indicated that the prolonged use of chemical fertilizers accelerated degradation of soil and a decline in crop productivity (Ram *et al*. 2016). An experiment conducted by Rajanna *et al*. (2011) in rice crop reported that the yield parameters was maximum recorded at treatment received inorganic supplemented with organic inputs.

According to IPCC climate change data from 2013, CO_2 emissions increased from 280 parts per million (1850) to above 400 parts per million (2019), which corresponds to a global mean temperature rise of 0.850 degrees Celsius between 1800 and 2012 . Soil organic carbon concentration of India is severely depleted, and is below the critical limits for soil and ecosystem functions (Lal 2008). SOC study is important because soil organic matter is a central soil property that heavily affected by management practices, which in turn influences soil physical, chemical and biological function.

Microbial and enzyme activity are major drivers for decomposition of organic matter and mineralization of nutrients. Soil microbial biomass typically accounts for 1-5 % of total soil carbon and can serve as an early warning system for the potential

degradation of soil quality caused by various management approaches (Mandal *et al*. 2007). Previous study showed that organic manures (FYM) along with recommended dose of fertilizers found to be viable option in increasing soil organic carbon, nutrient turn over, enhancing microbial biomass, there by improvement of availability of nutrients in soil, maintenance of soil quality and achieving the sustainable productivity of sorghum and wheat for long run in rainfed as well as irrigated moisture regimes (Katkar *et al*. 2011). The respiration rate per unit of microbial biomass or metabolic quotient has been utilized as a microbial stress indicator and interpreted as microbial efficiency. Hence, higher metabolic quotient was noticed in soil under nutrient-deficient stress than non-stressed soils (Fernandes *et al*. 2005). Soil enzymes would be used as an index to evaluate the effects of long-term nutrient management approaches (Balachandar *et al*. 2016).

With this background, the present study was carried out in the ongoing Permanent Manurial Experiment (PME) with long term nutrient management and continuous cropping in an *Alfisol* to evaluate the long term effect of inorganic fertilizers and manures on soil organic carbon and microbial activity. This location is generally acknowledged as the second oldest PME in the world, after Rothemstead, UK. The PME has been in existence for 112 years and has produced a dataset on soil physical, chemical, and biological features as they are subjected to a maize–sunflower cropping sequence in red sandy loam soil. In this study we hypothesized that: (1) combined application of chemical fertilizers and organic amendments would increase SOC content and crop yield to different degrees compared with chemical fertilization alone (2) long-term usage of organic and inorganic nutrients creates vital role in improving the microbial activity in the soil.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in on-going project at the century-old Permanent Manurial Experiment (PME) in Tamil Nadu Agricultural University (11°N, 77°E) Coimbatore, India. To study the long-term effect of nutrient management on crop yield and soil health after harvest of maize crop (172th crop). The climate of this site is Semi-arid to sub-tropical. The mean annual rainfall is about 674.2 mm with 34.3°C maximum mean annual temperature and 21.7°C minimum mean annual temperature. The cropping sequence followed is maize –sunflower having irrigated cropping situation. The soil is classified as *Typic haplustalfs* comes under Palathurai Soil series which is derived from sandy loam texture.

Treatment details

The experiment included two crops per year, sunflower (June-October) and maize (November-February). The treatments are T_1 Control (unfertilized and unmanured), T_2 100% NK, T_3 100% NP, T_4 100% NPK, T_5 Farmyard manure (FYM) N equivalent basis @ 50 t ha^{-1}, T_6 Farmyard manure (FYM) every year @ 12.5 t ha^{-1}, T_7 Poultry manure N equivalent basis @ 11.4 t ha^{-1}, T_8 100% NPK + Farmyard manure (FYM) @ 12.5 t ha^{-1} (INM). The hybrid maize CO 6 was sown during December 2021 and harvested during April 2022. The recommended dose of N, P_2O_5 and K_2O 250:75:75 kg ha^{-1} was applied to maize. The sources of N, P and K used were urea, single super phosphate and muriate of potash, respectively for all the treatments. For treatments T_6 well-decomposed farmyard manure (FYM) at 12.5 t ha^{-1} (fresh-weight basis) with an average nutrient composition of 0.5% N, 0.23% P and 0.53% K was broadcasted 20 days before sowing and mixed with soil.

Plant and soil sampling and analysis

Grain and straw yield of maize was recorded and expressed in kg ha^{-1}. Soil samples were collected from each plot after the harvest of maize crop. The samples were collected, air-dried, passed through a 2 mm mesh, and stored at 4°C. The subsamples were further ground to pass through a 0.25 mm mesh for SOC analysis. SOC was quantified by Walkley and Black wet digestion method (Walkley and Black 1934).

Soil biological characteristics were determined by gently sieving field moist soil samples through a 2 mm sieve. The soil respiration index was calculated

using the incubation and titration method (Anderson 1982). Weighted sieved field moist soil (10 g) was placed in an airtight 1 L capacity conical flask. A vial containing 20 ml of 1 M NaOH was left hanging, and the conical flask was air tightened. For 10 days, the soil was incubated at 28 ± 2ºC. The amount of carbon dioxide-carbon (CO_2-C) evolved and trapped in alkali was calculated by adding phenolphthalein and titrating with 0.5 M HCl. A saturated $BaCl_2$ solution was added prior to titration to precipitate the carbonates and bicarbonates as Barium carbonate.

Soil microbial biomass carbon (C_{mb}) in moist soil was determined immediately after sampling using fumigation extraction method (Vance *et al*. 1987). Chloroform fumigated soil (20g) and unfumigated soils (20g) were extracted with 100 ml of 0.5 M K_2SO_4. The C_{mb} in the extract was estimated after oxidation with 0.2 N $K_2Cr_2O_7$ at 100ºC for half an hour. C_{mb} in the extract was calculated using given formula

$$C_{mb} = (C_f – C_{uf}) K_{ex}^{-1}$$

Where C_{mb} is soil microbial biomass carbon in 1g soil, C_f and C_{uf} are 0.5 M extractable organic carbon in fumigated and unfumigated soils, respectively, K_{ex} is the efficiency of extraction, a value of 0.45 has been considered for calculation. The specific respiration rate (qCO_2) or metabolic quotient (respiration per unit of microbial biomass) was determined as a ratio of soil respiration index to soil microbial biomass carbon and reported as mg CO_2–C mg^{-1} C_{mb} day^{-1}.

Dehydrogenase activity of the soil was determined using the method by Tabatabai and Bremner (1969). Soil (l g) was poured in a 100 mL Erlenmeyer flask, and 0.2 mL triphenyltetrazolium chloride and 0.5 mL 1 % glucose solution were added. The tubes were then incubated for 24 hrs at 27ºC. Following the incubation period, 10 mL methanol was added, vigorously shaken, and allowed to stand for six hours. The absorbance at 485 nm was measured after the clear pink supernatant was removed. The dehydrogenase activity was measured in 1g Triphenylformazan (TPF) produced g^{-1} day^{-1}.

Alkaline phosphatase activity of the soil was estimated using the method by Tabatabai and Bremner (1969). Soil (1 g) was taken into Erlenmeyer flask and 0.2 ml toluene and 4 ml of modified universal buffer (pH 11) was added, followed by addition of 1ml of 0.5M p-nitro phenyl phosphate (pH 11) and swirled the flak for few seconds and then incubated for 1 hour at 37ºC. After incubation, 1 ml of 0.5M Calcium chloride and 0.5 M sodium hydroxide was added and again swirled for few seconds. Then the soil suspension was filtered through Whatman No.2 filter paper. The suspension was filtered and yellow color intensity of the filtrate was estimated at 420 nm wavelength. The alkaline phosphatase activity is expressed as µg PNP released g^{-1} h^{-1}.

Urease activity in soils was estimated as amount of urea hydrolyzed after incubation (Tabatabai and Bremner 1972). For 5 hrs, soil (5 g) was treated with a known amount of urea at 37°C. The suspension was filtered and 5 mL of the filtrate was mixed with potassium chloride-phenyl mercuric acetate, and also a coloring agent. The solution was heated in a hot water bath for half an hour and then allowed to cool. At a wavelength of 527 nm, the intensity of red color was detected. The urease activity was expressed mg urea hydrolyzed g^{-1} h^{-1}.

Total microbial population in the experimental and control plots were determined using serial dilution and plating technique (Parkinson *et al*. 1971). Fresh soil (1 g) was diluted in 10 ml water to get 10^{-1} dilution. After shaking 1 ml of dilution was taken and transferred to 9ml water blank to get 10^{-2} dilution. Likewise samples were diluted serially. Afterwards 1 ml of these samples were transferred on to soil extract agar medium plate in the following manner; 10^{-6} dilution for bacteria using nutrient agar media, 10^{-4} dilution by using Rose Bengal agar for fungi, 10^{-3} dilution using ken knight agar medium for actinomycetes. Colonies kept at 30ºC incubation were counted after 48 hrs, 5 days and 7 days for bacteria, fungi and actinomycetes respectively which expressed as cfu g^{-1} of soil dry weight basis.

RESULTS AND DISCUSSION

Crop yield

Application of fertilizer nutrients either alone or in

Table 1. Long-term effect of manure and inorganic fertilizer of grain and stover yield of maize in year 2021-2022.

Treat-ments	Treatment details	Maize grain yield (kg ha^{-1})	Stover yield
T_1	Control	935	1508
T_2	NK	3034	5064
T_3	NP	6415	6410
T_4	NPK	6475	9114
T_5	FYM (N Equivalent basis)	5167	8704
T_6	FYM (Every year)	3516	7640
T_7	Poultry manure	6173	8886
T_8	NPK+FYM	7786	12534
	Mean	4938	7483
	SEd	1.74	43.85
	CD (P= 0.05)	3.73	94.06

combination with FYM greatly influenced the grain and stover yield of maize. Generally, plots with any fertilization produced significantly higher crop yield than the unfertilized plots. Current year data also shows highest grain and stover yield for NPK+FYM compare to NPK and organic manures (Table 1).

Data on mean grain yield from 2008 onwards revealed that continuous application as 100%NPK+ FYM @12.5 t ha^{-1} achieved highest yield every year (Fig. 1). Sustained soil fertility by repeated addition of FYM and NPK fertilizers and effective utilization of applied nutrients which increase sink capacity and nutrient uptake by maize. Treatments received only organics every year (FYM @ 12.5 t ha^{-1}) showed 50 % reduction in grain yield when compared to NPK. Unbalanced and organic manure alone applied plot did not result in better grain and stover yield compare to NPK and NPK+FYM plot. Quick availability of inorganic fertilizers and slow release of nutrient from FYM gives availability of nutrients during complete growth period and thereby NPK+FYM recorded higher yield. Similar result was also reported by Meena *et al.* (2019).

Soil organic carbon content

Change in soil organic C content was observed after 112 years of NPK+FYM treatment from 3.2 g kg^{-1} (1974) to 9.10 g kg^{-1} (2022) which indicated the build-up of SOC from base level. Likewise in 1953 organic carbon status in FYM treatment was 1.6g kg^{-1} and NPK treatment was recorded 1.4 g kg^{-1} (Santhy and Devarajan 2018) which increase to 7.25 g kg^{-1} In FYM treatment and 6.31g kg^{-1} in NPK treatment in the year 2022. Over the years high increase in SOC content was observed in NPK +FYM treatment plot (Fig. 2). Use of FYM stimulates the microbial activity which resulted in enhanced polysaccharides production and stabilization of organic matter in the soil which is attributed the higher SOC. Similarly organic manure applied plot shows high SOC compare to NPK .

Continuous application of FYM alone @ 12.5 t

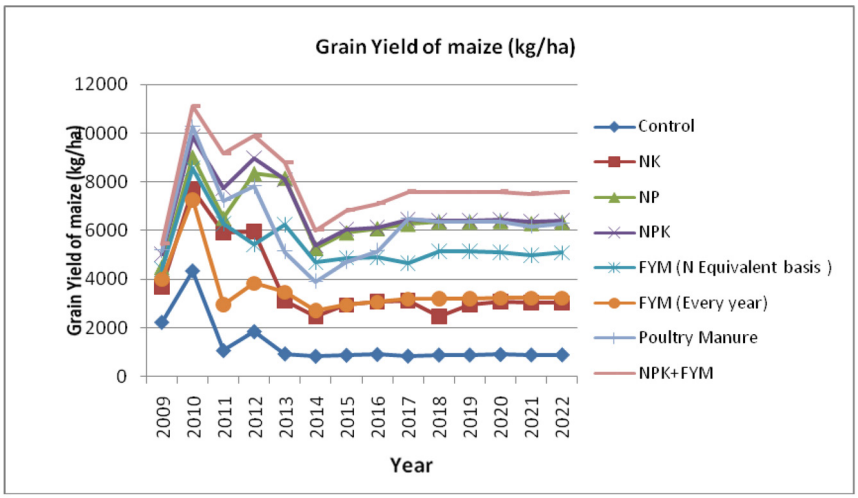

Fig. 1. Effect of continuous fertilization on maize yield in PME field.

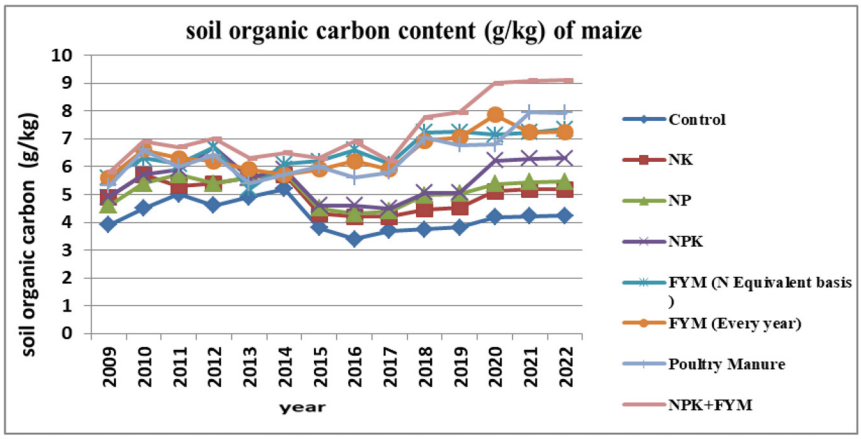

Fig. 2. Long-term effect of manure and inorganic fertilizer on organic carbon content in PME field.

ha^{-1} over 112 years had 15% higher SOC over NPK treatment and 71 % higher over control. Treatment received NPK alone had 49% more SOC than control which might be due to enhanced root residue addition to the soil under continuous cultivation (Fig. 2). This is in conformity with the findings of (Li *et al*. 2013) who reported that the balanced fertilization enhanced SOC content compared to unbalanced fertilization. The lowest SOC content was noticed in control plot (4.23 g kg^{-1}). Similar findings were reported in the long-term experiment in an Inceptisol by Arulmozhiselvan in the year 2013 and he stated that the highest organic carbon content was recorded under 100% NPK + FYM also continuous application of graded levels of NPK from 50% to 150% was accomplished by a corresponding increase in the organic carbon content and control and 100% N treatments recorded the lowest organic carbon.

Table 2. Microbial biomass carbon, CO_2 evolution index and metabolic quotient in PME in the year 2022.

Treat-ments	Treatment details	Microbial biomass carbon (mg/kg)	CO_2 evolution index (mg CO_2 in 100 g soil)	Metabolic quotient (qCO_2)
T_1	Control	163	35.1	0.215
T_2	NK	187	39.6	0.211
T_3	NP	206	41.8	0.202
T_4	NPK	223	44.2	0.198
T_5	FYM (N Equiv-alent basis)	290	50.17	0.173
T_6	FYM (Every year)	284	49.76	0.175
T_7	Poultry manure	295	50.7	0.171
T_8	NPK+FYM	325	52.8	0.162
	Mean	246	44.6	0.188
	SEd	1.44	0.13	0.0034
	CD (P = 0.05)	3.08	0.28	0.0073

Soil microbial activities

In the present investigation the microbial biomass carbon (MBC) was significantly affected by long-term addition of nutrients. Integrated application of NPK + FYM recorded the highest MBC content (325 g kg^{-1}) and control plot recorded lowest (163 g kg^{-1}).The plot supplied with FYM alone recorded higher MBC content (284 mg kg^{-1}) when compared to the plot supplied with optimum NPK (223 mg kg^{-1}). The microbial biomass carbon (MBC) content of the NPK+FYM increased by 45% over the NPK treatment (Tables 2-3). Apart from increased root biomass, the exudates and mucilaginous substance produced by roots might result in increased MBC (Dutta *et al*. 2015).

Normally, the microbial biomass carbon makes up only a small fraction of SOC (1-5%). It is more dynamic and unstable than the SOC or SOM over a short period of time. As a result, in addition to measuring SOC, microbial biomass carbon could be an excellent indication for judging changes owing to management methods (Ghosh *et al*. 2018). Similar findings were reported by Xu *et al*. (2018) in clay loam soil under wheat based cropping system con-

cluded that, ratio of soil microbial biomass carbon (SMBC) to soil microbial biomass nitrogen (SMBN) was higher in treatment received NPK combined with organic source such as pig manure, straw return, pig manure plus straw return. This shows that the organic manure along with chemical fertilizers promote growth and reproduction of microbial biomass and therefore resulted in higher microbial community structure and SMBC/SMBN ratio.

Respiration rate is considered to reveal the availability of carbon for microbial maintenance and is a measure of basic turnover rates in soils (Insam *et al*. 1991). It acts as an indicator of microbial activity and is influenced by availability of carbon to microbes in soil environment. The soil respiration was significantly varied between treatments. It ranged between 35.1 mg CO_2 100 g^{-1} soil in control and 52.8 mg CO_2 100 g^{-1} soil in NPK+FYM (Table 3). The highest CO_2 evolution was found in NPK+FYM and lowest CO_2 evolution was noticed in control plot. All other treatments were comparable with each other.

The specific respiration rate (qCO_2) or metabolic quotient (respiration per unit of microbial biomass) is a valuable factor of bio energetic changes in developing ecosystem (Ep 1969). It also reflects metabolic efficiency of microbial community. The metabolic quotient (qCO_2) of the soil was significantly influenced by continuous application of fertilizers and manures over 112 years. The treatments which received FYM continuously such as NPK+FYM and FYM showed significantly lower qCO_2 of 0.162, 0.175 respectively as compared to other treatments (Table 2).

The low qCO_2 suggests a more effective utilization of substrates by the soil microbes. Higher qCO_2 levels suggest that the microbial population and microorganisms are under stress and they must consume more C for maintenance rather than growth (Anderson and Domsch 1993).

Soil enzymes

Organic (FYM alone) and NPK+FYM treated plots had significantly higher urease activity than control and inorganic plots. The enhanced levels of urease in FYM received plots might be due to continuous availability of substrates for the enzyme either in the form of organic sources (nitrogenous substance produced by root exudates) or organic plus inorganic sources (N from applied urea) (Elayaraja and Singaravel 2011). Low level of urease activity in inorganic fertilizer treated soil as compared to organic and INM treatments (35% higher over NPK) indicated that mineral fertilization without a sufficient amount of available organic substrate may not have an impact on urease activity (Zaman *et al*. 2008).

Soil dehydrogenase (DHA) activity is a useful indication of soil microbial activity because it shows the whole range of oxidative activity and viable microbial populations (Nannipieri *et al*. 2003).In the present study, compared to NPK and FYM alone treated plots, the integrated application of NPK+FYM showed higher DHA activity of about 45 and 20 % respectively. Application of FYM alone had 21 % higher DHA activity than NPK treated plot (Table 3). The highest dehydrogenase activity in treatments applied with FYM may be attributed to FYM which might have provided diversified, more complex nutrient amendments and a suitable environment for more accumulation of enzymes in soil matrix (Rout and Pragyan 2017). Soil dehydrogenase (DHA) activity indicates the functions of total range of oxidative activity and viable microbial communities, serves as a good indicator of soil microbial activity (Nannipieri

Table 3. Enzyme activity in PME in the year 2022.

Treatments	Treatment details	Urease (µg of NH_4 released $g^{-1}h^{-1}$)	Dehydrogenase (µg TPF g^{-1} day^{-1})	Alkaline phosphatase (µg PNP $g^{-1}h^{-1}$)
T_1	Control	26.7	4.33	26.09
T_2	NK	39.6	7.32	34.56
T_3	NP	37.5	7.58	35.58
T_4	NPK	43.4	8.07	41.97
T_5	FYM (N Equivalent basis)	48.9	9.83	47.37
T_6	FYM (Every year)	46.8	9.74	47.14
T_7	Poultry manure	51.8	9.92	51.35
T_8	NPK+FYM	58.7	11.73	59.01
	Mean	44.2	8.57	42.88
	SEd	0.1	0.04	0.03
	CD (P=0.05)	0.3	0.09	0.06

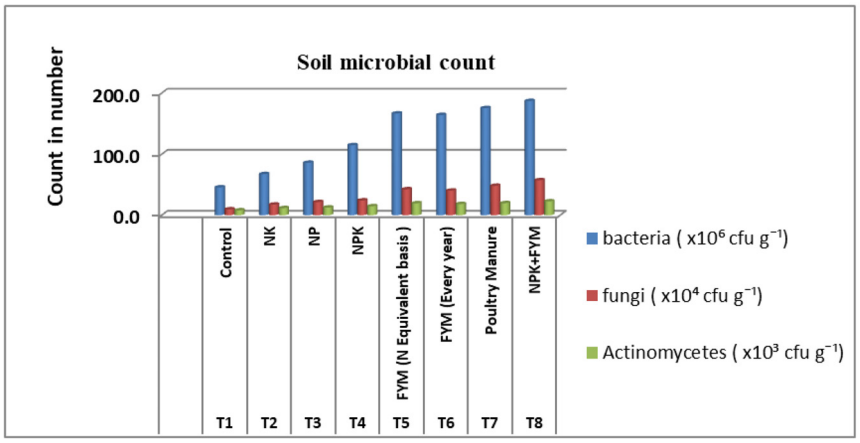

Fig. 3. Comparison of different microbial population in different treatment plots in PME in the year 2022.

et al. 2003).

Integrated application of inorganics and organics as NPK+FYM recorded the highest ALP activity (41% higher over NPK). The enhanced ALP activity in treatments receiving FYM either alone or with inorganics might be due to the improved microbial activity and perhaps multiplicity of phosphate solubilizing bacteria due to manure input over ten consecutive years (Ramdas *et al*. 2017). Addition of organic manures in NPK and FYM acts as nutrients supplement and affords as source of enzyme as well as substrates for hydrolysis (Balachandar *et al*. 2016).

First, soil enzymes are produced mainly by soil microorganisms and dead plants and animals and the supply of organic materials increases C sources for soil microorganisms. This increases the energy available to them, which promotes their metabolism and reproduction, which thereby promotes the increase in soil enzyme activity (Ma *et al*. 2012). Second, the organic matter improves the physico-chemical properties of soil, providing a better environment for the growth of microorganisms and soil fauna. The decomposition of organic matter quickens, which in turn provides more substrate for soil enzymes and thereby induces an increase in soil enzyme activity (Timo *et al*. 2006).

Soil microbial count

The population of bacteria, fungi, actinomycetes present in soil samples showed that the control plot recorded lower population than other treatments. Plot treated with NPK+FYM recorded higher microbial population in surface layer. Among microbes bacterial population was highest compare to the fungi and actinomycetes. In soil over 112 years of continuous fertilization. Microbial population helps in mobilizing native nutrients in soil plant system, providing favorable C:N ratio for higher activity of microbes and leads to higher microbial biomass carbon accumulation in soil. The maximum bacterial population (187 x10^6 cfu g^{-1}) and fungal population (57.3 x10^4 cfu g^{-1}) were observed in NPK+FYM while control plot recorded minimum bacterial (45.6 x10^6 cfu g^{-1}) and fungal population (9.7 x104 cfu g^{-1}). Among all treatments combined application of NPK + FYM recorded highest actinomycetes count (Fig. 3).

In present study it is observed that FYM improves microbial communities in soil. This may be due to applied manure which up on decomposition acted as source of substrate for microbial growth. The optimum NPK application did not cause drastic reduction in counts of microbial communities compared to the control plot. Similar findings were also reported by Balachandar *et al.* (2016). Dong *et al*. (2014) reported a significant change in bacteria, actinomycetes and fungi population in soils which are subjected to organic manure applications, than in soils treated with mineral fertilizer. The counts of total bacteria, fungi and actinomycetes were significantly higher in balanced manure and mineral fertilizers

than those devoid of any nutrient from treatments, which revealed that the lack of any individual nutrient element decreased total soil microbial populations (Naher *et al*. 2013).

CONCLUSION

The present investigation concludes that application of FYM along with inorganic fertilizers improve over the years crop yield, soil organic carbon content, and biological properties of the soil and hence could be adopted for maintaining the soil fertility in order to sustain soil productivity over long run. Compared to inorganic treatment (NPK), organic plot (FYM) recorded the highest biological properties (Microbial population, Microbial Biomass Carbon, CO_2 evolution and Enzymatic activities). While, the lowest biological properties were observed under control plot. Microbial communities show positive correlation with SOC build up and microbial biomass carbon. The low metabolic quotient (respiration per unit of microbial biomass) or specific respiration rate (qCO_2) suggests a more effective utilization of substrates by the soil microbes. With regard to soil enzymes, enhanced urease and dehydrogenase activity were found in NPK+FYM plot. Soil microbial population was highest in the order of bacteria> fungi> actinomycetes. Therefore, judicious application of inorganic and organic nutrients in an integrated manner is essential for proper nutrient supply and sustaining crop productivity in an intensive cropping system and maintaining the soil health.

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