ISSN: 1394-7990
Malaysian Journal of Soil Science Vol. 20: 147-162 (2016) Malaysian Society of Soil Science

Impact of Organic Manure and Inorganic Fertiliser on Soil Enzymatic Activity and Microbial Diversity in the States of Tamil Nadu and Madhya Pradesh, India

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ABSTRACT

A laboratory study was conducted to examine the effects of organic and inorganic cultivation on soil biological processes and biodiversity. Five soil samples from each of the organic manure treated fields and inorganic fertiliser treated fields from two different states, Tamil Nadu and Madhya Pradesh, in India were examined. The soil types were either black cotton or loamy soil. Two other soil samples from a fallow area of Indore, Madhya Pradesh, India were also included for nutrient status and biodiversity comparison. Soil organic carbon, nitrogen, phosphorus, and potassium levels and soil enzymes that reflected soil microbial activity, such as dehydrogenase, beta glucosidase, phosphatase and nitrate reductase, were estimated. Inorganic fertilizer treated soils had the lowest organic carbon content (4.5 g kg^{-1}) compared to the highest (12.2 g kg^{-1}) in organic manure treated soils. Similarly, soil phosphatase, glucosidase and dehydrogenase activities were higher by 26%, 28% and 21%, respectively, in organic fertilizer treated soils. Randomly Amplified Polymorphic DNA (RAPD) profiles of soil DNA indicated microbial richness in organic manure treated soil as it had a low Jaccard's similarity coefficient of 0.577 vs 0.703 in inorganic fertilizer treated soil. Soil microbial diversity and dynamics were found to be greater in the organic system of cropping. These findings suggest that these could be used as potential indicators for soil health.

Keywords: Microbial dynamics, RAPD, soil enzymatic assay, farm yard manure, inorganic fertiliser

INTRODUCTION

India is the third largest consumer of fertilisers, next to China and USA, in the world. Fertiliser consumption of India was 24.48 million tons during the 2013-14 period (Indian Fertiliser Scenario, 2014). Besides the application of fertilisers, considerable amounts of agrochemicals such as pesticides and herbicides are also used. Use of chemicals at such scale causes environmental pollution, deteriorates soil health and agro-ecology, and leads to poor profitability in farming (Robertson and Swinton, 2005). This has basically prompted the demand for organic cultivation for conservation and optimised utilisation of all natural resources (Mader *et al*., 2002). In addition, at present organic produce generally

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command a higher price than conventional produce (Oberholtzer *et al*., 2005). Organic farming results in produce that is higher in dry matter content, higher mineral concentrations, lower nitrate (NO_3) concentrations, higher vitamin C concentrations, higher phytonutrient content, and better taste (Rosen and Allen, 2007).

Nutrients are a controlling input to the soil system and the processes within it. Carbon content, cycling of nitrogen and phosphorus affect soil dynamics and agricultural production (Barber, 1995). Changes in soil organic carbon and nitrogen are reflective of crop rotation and fertiliser addition (Omay *et al*., 1997). Long-term inorganic N application decreases organic matter and biological activity, whereas short-term inorganic N application has limited effects on soil enzyme activities and microbial biomass C (Fauci and Dick, 1994). An increase in soil microbial population viz., bacteria, fungi and actinomycetes, was observed with the application of organic N sources (Krishnakumar *et al*., 2005) compared to the inorganic form. Microbial community structure and its activity in soil are indicators of soil quality and plant productivity (Latour *et al*., 1996). Soil enzyme quantities are suitable indicators of soil quality because they are the measure of soil microbial activity (Dick *et al*., 1996). Soil enzymes such as protease, glucosidase and alkaline phosphatase were found to directly correlate with the microbial biomass carbon, microbial biomass nitrogen, growth and activity (Melero *et al.*, 2008). An increase in dehydrogenase activity after the incorporation of organic carbon input was observed by Parham *et al.*, (2002) and Madejon *et al*., (2007). The present study was carried out to endorse the effects of organic manure application on soil quality and microbial diversity.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples from purely farm yard organic manure applied fields, inorganic fertilisers and chemicals treated fields and fallow land were collected from Coimbatore District in Tamil Nadu and Indore in Madhya Pradesh State of India. Soil samples were collected randomly at five locations at a depth of 15 cm using a spade. The samples were pooled and composited, processed and sieved through a 2-mm pore sieve prior to the analysis. In all the cases, sampling was done at the end of the cropping season. Twelve composite soil samples were collected from five organic farming fields (O1, O2, O3, O4, O5) five from inorganic (I1, I2, I3, I4, I5) fields and two from fallow land (F1, F2). Organic farming fields were fields that strictly used organic manure in the form of farm yard manure with no application of chemical pesticides and fertilisers. Farm yard manure composition is mainly cow dung. Soil samples taken for the study were visually examined and sieved for removal of debris. The crops grown and the soil type of the respective fields are listed in Table 1. All soil samples were used for soil metagenomic analysis and nutrient content estimation whereas only four among five organic and inorganic soil samples were used for soil enzyme activity assays.

TABLE 1
Soil types used in this study, their location and cultivation practices

S. No.	Soil Sample	Farming System	Soil Type	Location	Crop
1.	F1	Fallow soil	Black cotton soil, heavy	Badia Kema, Indore	Fallow soil
2.	F2	Fallow soil	Black cotton soil, light	Badia Kema, Indore	Fallow soil
3.	I1	Inorganic fertilizers and chemicals	Black cotton soil, heavy	Badia Kema, Indore	Soya bean
4.	I2	Inorganic fertilizers and chemicals	Black cotton soil, light	Badia Kema, Indore	Soya bean
5.	I3	Inorganic fertilizers and chemicals	Black soil	Coimbatore	Tomato
6.	I4	Inorganic fertilizers and chemicals	Black soil	Coimbatore	Chilly
7.	I5	Inorganic fertilizers and chemicals	Loamy soil	Coimbatore	Maize
8.	O1	Only organic manure used in farming	Black cotton soil, heavy	Badia Kema, Indore	Soya bean
9.	O2	Only organic manure used in farming	Black cotton soil, light	Badia Kema, Indore	Soya bean
10.	O3	Only organic manure used in farming	Black soil	Coimbatore	Tomato
11.	O4	Only organic manure used in farming	Black soil	Coimbatore	Chilly
12.	O5	Only organic manure used in farming	Loamy soil	Coimbatore	Maize

Determination of Soil Nutrients

Total nitrogen and phosphorus in soil samples were estimated by the method described by Pape *et al*., (1982). Soluble phosphorus was extracted by the addition of 0.03 N ammonium fluoride and 0.025 N hydrochloric acid. Ammonium molybdate and stannous chloride were added to the extracted solution after filtration with Whatman No 41 filter paper. The spectrophotmetric quantification of phosphorus was carried out by measuring the characteristic blue colour solution at 690 nm (UV-1601, Shimadzu). Total nitrogen was determined by Kjeldahl method. Soil samples were digested with concentrated sulphuric acid in the presence of copper sulphate and potassium sulphate. The ammonia evolved during digestion was collected as distillate with hydrochloric acid as absorbent. The excess acid was titrated with sodium hydroxide solution to determine ammonia/nitrogen content. The potassium content in the soil was determined by flame photometer (Elico Flame photometer CL 378, India). Potassium chloride was used as standard for potassium. The potassium in soil was extracted with solution of 0.5M ammonium acetate and 0.5M acetic acid for 30 min. The organic carbon content of soil was done according to the Walkley and Black procedure

with minor modification (Hooda and Kaur, 1999). Briefly this procedure involved the addition of concentrated sulphuric acd and potassium dichromate to the soil with the mixture being boiled at 150° C for 30 min. After cooling, the excess $Cr_2O_7^{2-}$ was titrated with ferrous sulphate to determine the organic carbon content.

Dehydrogenase Assay

Dehydrogenase activity was measured by the procedure described by Pepper *et al.* (1995). Briefly, this procedure utilised triphenyltetrazolium chloride (TTC) as the electron acceptor, which was reduced to red-colored, methanol-soluble, triphenyl formazan (TPF). Six g of moist soil from each sample was placed in a test tube and incubated statically with 1 mL of 3% TTC (3 g /100 mL deionised water) and 3 ml of 0.2 M $CaCO_3$ buffer solution for 24 h at 37°C. This reaction was terminated by 10 ml of methanol, and TPF was extracted with 30 ml of additional methanol. The final extract was filtered with Whatman No.42 filter paper and TPF concentration was determined spectrophotometrically at 485 nm.

Beta -Glucosidase Assay

Beta-glucosidase activity was quantified according to procedures described by Tabatabai (1994). The method is based on colorimetric measurement of p-nitrophenol released by â-glucosidase when soil is incubated with buffered (pH 6.0) p-nitrophenyl-ß-glucopyranoside solution. Two g of moist soil sample was incubated with 50 mM acetate buffer pH 5, for 1 h at 37°C. This solution was filtered using Whatman No. 42 filter paper. An aliquot of 750 µL of sample was mixed with 750 µL of 5 mM para nitrophenyl-B-glucopyranoside. This was incubated for 1 h at room temperature. Para nitrophenol released by the action of B-glucosidase on the substrate gave a yellow colour solution. The absorbance was measured at 410 nm. A standard curve was prepared with dilutions of a para nitrophenol in buffer solution.

Phosphatase Assay

The method is based on colorimetric measurement of p-nitrophenol released by phosphatase when soil is incubated with buffered (pH 6.0) p-nitrophenyl phosphate (PNG) solution. Two g of moist soil sample was incubated with 50 mM acetate buffer pH 5 for 1 h at 37°C. This solution was filtered using Whatman No.42 filter paper. An aliquot of 750 µL of sample was mixed with 750 µL of 5 mM para nitrophenyl phosphate. Again the solution was incubated for 1 h at room temperature. Para nitrophenol released by the action of phosphatase on the substrate gave a yellow colour solution. The absorbance was measured at 410 nm using UV-visible spectrophotometer (UV-1601, Shimadzu). A standard curve was made with dilutions of a para nitrophenol in buffer solution.

Nitrate Reductase Assay

The method is based on colorimetric measurement of nitrite colour complex formed when nitrite is formed as a result of nitrate reductase activity when it combines with

sulphanilamide and N-(1-Naphthyl) etylenenediamine dihydrochloride. Two g of moist soil sample was incubated with 50 mM, acetate buffer pH 5 for 1 h at 37°C. A volume of 1.9 mL of sample extract was mixed with 100 µL of 2 mM reduced nicotinamide adenine dinucleotide and incubated at 300C for 2 min. Then, 1 ml of 58 mM sulphanilamide solution and 1 mL of 0.77 mM napthyl- ethylenediamine dihydrochloride solutions were added. This mixture was incubated for 10 min at 25^0C after thorough mixing. Absorbance was checked for the sample at 540 nm using UV-visible spectrophotometer (UV-1601, Shimadzu). Standard curve was prepared using sodium nitrite dilutions.

Isolation of DNA from Soil

Soil DNA was isolated using Power Soil DNA isolation kit (MO BIO, Laboratories. Inc.). The DNA isolation procedure was according to the manufacturer's protocol. Soil sample taken for isolation of DNA was 0.25g and the DNA was dissolved in 100µL TE and stored at -20°C.

DNA Quantification

To evaluate the purity and concentration of the extracted DNA, absorbance ratios at 260 nm/230 nm (DNA / humic acids) and 260 nm/280 nm (DNA / protein) were determined (Sambrook *et al.*, 1989) using UV-visible spectrophotometer (UV-1601, Shimadzu).

RAPD PCR Amplification

The random amplification of polymorphic DNA (RAPD) technique is a PCR-based method that uses a short primer (usually 10 bases) to amplify anonymous stretches of DNA. The list of random primers used in the experiment is given in Table 2. They are custom made from Sigma-Aldrich.

TABLE 2
Random primers used for RAPD amplification of the soil DNA in this study

S.No	PRIMER	SEQUENCE (5'-3')	REFERENCE
1.	OPG-2	GGCACTGAGG	Nimisha *et al.*,2008
2.	OPG-3	GAGCCCTCCA	Nimisha *et al.*,2008
3.	OPG-11	TGCCCGTCGT	Nimisha *et al.*,2008
4.	OPG-12	CAGCTCACGA	Nimisha *et al.*,2008
5.	OPG-14	GGATGAGACC	Nimisha *et al.*,2008
6.	OPG-16	AGCGTCCTCC	Nimisha *et al.*,2008
7.	PS01	CGTCACAGAG	Yang *et al.*,2000
8.	PS02	GAGGCCCGTT	Yang *et al.*,2000
9.	PS03	CAGGCTCTAG	Yang *et al.*,2000
10.	PS04	GTTGTGCCTG	Yang *et al.*,2000

RAPD-PCR was performed in a final volume of 25 µL containing 10X assay buffer, 1.0 unit of Taq DNA polymerase, 200 µM each of dNTPs, 10 pmol/ reaction of random primers and 50 ng template DNA. A thermal cycler was programmed for the initial denaturation step (94°C) of 5 min, followed by 45 cycles of 1 min denaturation along with 1 min primer annealing (37°C) and 2 min primer extension (72°C), followed by a final 7 min primer extension (72°C) step. Amplified fragments were resolved by electrophoresis on 1.2% agarose gels.

RAPD Data Analysis

The RAPD profiles of the various soil samples for the 10 random primers were scored. Presence of amplified fragment of particular size was scored as 1 and 0 for the absence of the same size fragment. The data was used to calculate the Jaccard's coefficients. Jaccard's coefficient is a measure of the similarity between sample sets, and is defined as the size of the intersection divided by the size of the union of the sample sets. These values were used to draw a dendrogram using UPGMA method.

Statistical Analysis of Data

Data obtained for soil nutrients and enzymes were statistically analysed, with the exception of DNA content. One-way ANOVA was performed using Dunnett's method and the level of significance is indicated in the respective tables and figures. Since organic manure and inorganic fertiliser treated soils were compared for differences without control, significance was calculated comparing the first I1 samples, hence level of significance will not be indicated in I1.

RESULTS

Comparison of Soil Nutrient Status

Total organic carbon content of soils treated with inorganic fertilisers was relatively low, ranging from 4.5-8.3 g kg^{-1} soil (Table 3). In contrast, soil samples treated with organic manure had a maximum of 12.2 g kg^{-1} soil of organic carbon (Table 3) in O5. The least amount of 4 g kg^{-1} soil of organic carbon content was found in fallow soil sample F2 (Table 3). The total nitrogen contents of soil samples followed the same trend as that of carbon. The nitrogen content of organic manure treated soil samples was the highest (1.39-1.68 g kg^{-1} soil), followed by inorganic fertiliser treated soil samples (0.99-1.5 g kg^{-1} soil) and fallow soil samples (0.77-0.91 g kg^{-1} soil) (Table 3). Among the organic manure applied soils, the phosphorus content was highest in O5 with 21.2 mg kg^{-1} soil (Table 3) whereas it was minimal in I5 (7.0 mg kg^{-1} soil) and only 3.8 and 2.7 mg kg^{-1} soil were found in F1 and F2, respectively (Table 3). Potassium content was observed to be maximum for an organic soil sample O1 (285 mg kg^{-1} soil), whereas the inorganic soil sample I4 recorded the minimum of 70 mg kg^{-1} soil which is even lower than the fallow soil sample of F2 which had 98 mg kg^{-1} soil (Table 3).

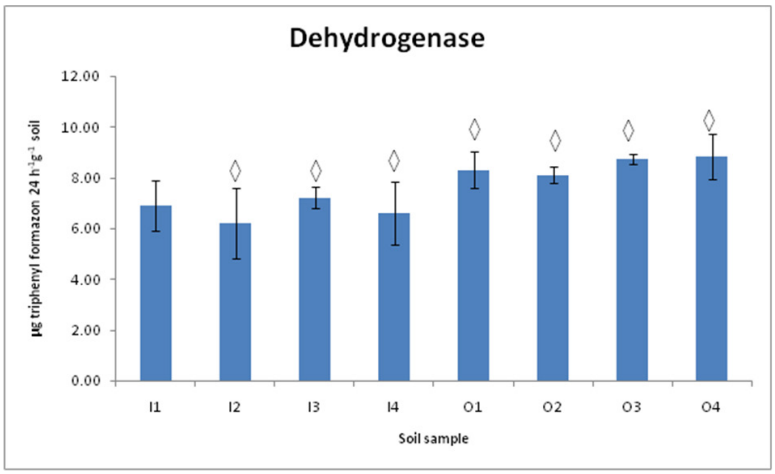
TABLE 3
Comparison of soil carbon and major nutrient levels in fallow, organic manure applied and inorganic fertiliser applied soil samples.

S.No	Nutrient	F1	F2	I1	I2	I3	I4	I5	O1	O2	O3	O4	O5
1.	Organic Carbon ($g\ kg^{-1}$)	4.40	4.00	4.80	4.50 ◊	8.30 **	7.50 **	7.00 **	9.60 **	8.10 **	12.20 **	10.90 **	11.29 **
2.	Total Nitrogen ($g\ kg^{-1}$)	0.91	0.77	1.04	0.99 ◊	1.23 ◊	1.50 **	1.24 ◊	1.42 **	1.39 *	1.68 **	1.53 **	1.61 **
3.	Phosphorus ($mg\ kg^{-1}$)	3.80	2.70	12.00	10.00 ◊	7.00 **	14.40 ◊	11.20 ◊	15.50 *	14.10 ◊	21.20 **	13.90 ◊	17.10 **
4.	Potassium ($mg\ kg^{-1}$)	136	98	173	149 ◊	145 ◊	98 **	70 **	285 **	197 ◊	235 **	141 ◊	146 ◊

◊ P>0.05 * P<0.05 ** P<0.01

Soil Enzyme Activities

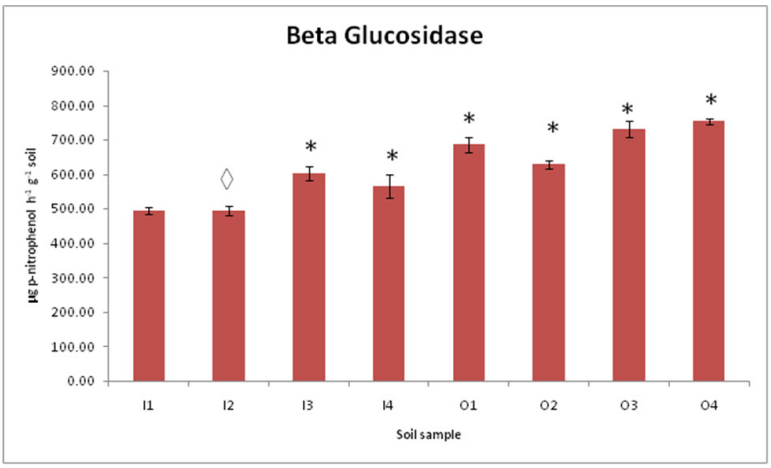
Dehydrogenase activity was found to be lower in soils treated with chemical fertilisers (Figure 1) with a range of 6-7 µg triphenyl formazon 24 $h^{-1}g^{-1}$soil. The organic manure treated soils showed a higher activity of ≥8 µg triphenyl formazon 24 $h^{-1}g^{-1}$soil (Figure 1). Similarly all organic manure treated soil samples had higher β-glucosidase activity than the chemical fertiliser treated soil samples (Figure. 2). The phosphatase activity was found to be much lower in inorganic fertiliser treated soil samples with a minimum of 320 µg p-nitrophenol h^{-1} g^{-1} soil in I2 in comparison with organic soil samples which showed the highest activity in O4 with 620 µg p-nitrophenol h^{-1} g^{-1} soil (Figure 3). The nitrate reductase activities of all inorganic soil samples were lower than in organic soil samples except for I3 (Figure 4).



◊ **P>0.05**

I1,I2,I3,I4 refer to soil samples from inorganic fertilizer treated farm and O1,O2, O3 ,O4 refer to soil samples from organic manure treated farms . ◊ Symbol indicates non significant values. Vertical lines on each bar indicate the error observed between replications.

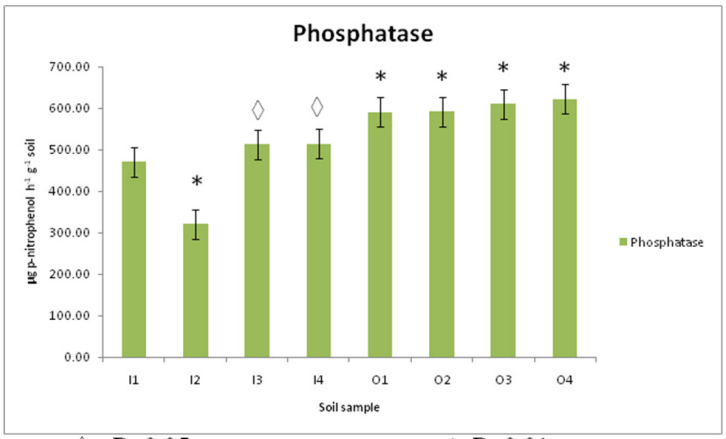
Figure 1: Comparison of dehydrogenase enzyme activity observed in organic manure and inorganic fertiliser treated soil samples



◊ **P>0.05** * **P<0.01**

I1,I2,I3 and I4 refer to soil samples from inorganic fertiliser treated fields and O1,O2, O3 ,O4 refer to soil samples from organic manure treated fields. ◊ indicates non significant values, * indicates significance at 0.01 level. Vertical lines on each bar indicate the error observed between replications.

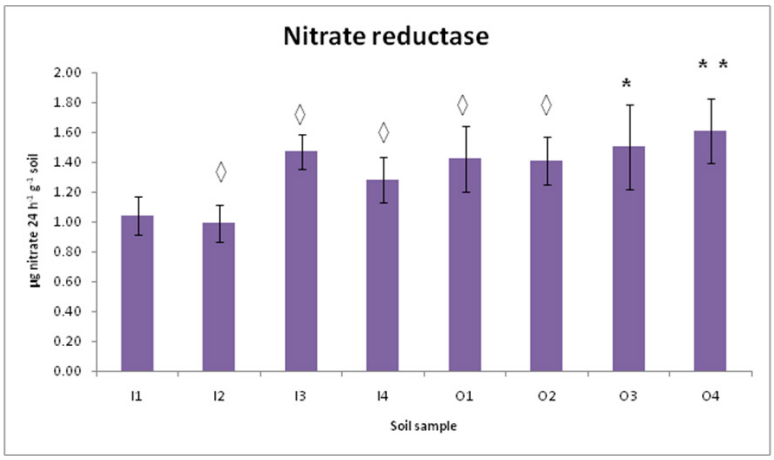
Figure 2: Comparison of betaglucosidase enzyme activity observed in organic manure and inorganic fertiliser treated soil samples



◊ P>0.05 * P<0.01

I1,I2,I3,I4 refer to soil samples from inorganic fertilizer treated farm and O1,O2, O3 ,O4 refer to soil samples from organic manure treated farms. ◊ Symbol indicates non significant, * indicate significance at 0.01 level. Vertical lines on each bar indicate the error observed between replications.

Figure 3: Comparison of phosphatasee enzyme activity observed in organic manure and inorganic fertiliser treated soil samples.



◊ P>0.05 * P<0.05 ** P<0.01

I1,I2,I3 and I4 refer to soil samples from inorganic fertiliser treated fields and O1,O2, O3 ,O4 refer to soil samples from organic manure treated fields . ◊ indicates non significant values, * indicates significance at 0.05 level, and ** indicate significance at 0.01 level. Vertical lines on each bar indicate the error observed between replications.

Figure 4: Comparison of nitrate reductase enzyme activity observed in organic manure and inorganic fertiliser treated soil samples.

DNA Content

Soil DNA concentrations were found to be higher in all the organic manure treated soil samples (Table 4) in comparison with inorganic fertelizer treated soil samples. Soil sample O4 recorded a maximum concentration of 5.34 mg of DNA per kg of soil. The percentage difference in DNA yield between organic and inorganic fertilizer treated soil was found to be highest in the loamy soil sample from Coimbatore.

RAPD Profile of Soil DNA

The microbial diversity of soil DNA was assessed by performing RAPD analysis on DNA isolated from soil samples collected from organic and inorganic fertiliser treated soil and fallow soil. The RAPD PCR profile for random primer OPG3 (Plate I) indicates a few fragment amplifications in fallow soil samples F1 and F2 (Lanes 2 and 3) and inorganic soil samples I1-I5 (Lanes 4,5,6,7 and 8).

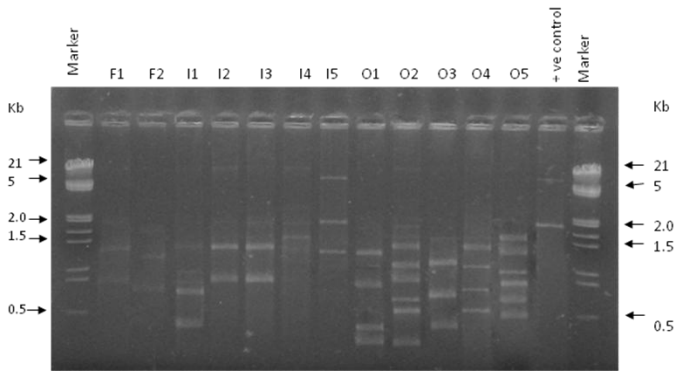
Plate 1. DNA amplification profile of a RAPD PCR using OPG3 primer on DNA isolated from soil samples from fallow land, organic manure and inorganic fertiliser treated farms. Lanes 1 and 15 are DNA markers, (lambda double digest), Lanes 2 and 3 correspond to soil samples F1 and F2, lanes 4,5, 6,7 and 8 are soil samples I1, I2, I3, I4 and I5, lanes 9, 10, 11, 12 and 13 are soil samples O1, O2, O3, O4 and O5, lane 14 is positive control for PCR

TABLE 4

Comparison of soil DNA content isolated from fallow, organic manure treated and inorganic fertiliser applied soil samples.

S.No.	Soil type location and Crop grown	DNA concentration(mg/kg soil) Inorganic fertilizer treated soil samples		DNA concentration (mg/kg soil) Organic manure treated soil samples		% difference [a]
1.	Black cotton soil, Heavy, Indore - Soybean	I1	4.83	O1	5.22	8.00
2.	Black cotton soil, light Indore - Soybean	I2	4.00	O2	5.16	29.00
3.	Black cotton soil, Coimbatore -Tomato	I3	4.02	O3	4.80	19.40
4.	Black cotton soil, Coimbatroe - Chilly	I4	4.49	O4	5.34	18.93
5.	Loamy soil Coimbatore - Maize	I5	3.80	O5	5.22	37.36
6.	Fallow black cotton soil, Heavy - No crop	3.23 mg/kg soil				
7.	Fallow black cotton soil, light - No crop	3.61 mg/kg soil				

[a] Difference in DNA yield between organic manure treated and inorganic fertiliser treated soil samples



Lanes 1 and 15 are DNA marker,(lambda double digest), Lanes 2 and 3 correspond to soil samples F1 and F2, lanes 4,5, 6,7 and 8 are soil samplesI1,I2,I3,I4 and I5, lanes 9,10,11,12, and 13 are soil samples O1,O2,O3, O4 and O5, lane 14 is positive control for PCR

The amplified polymorphic fragments in these lanes were not only few in number but also very faint compared to organic soil samples O1-O5 (lanes 9,10,11,12 and 13). A higher number of bands with more intense DNA bands was seen in the organic soils (lanes 9-13). The same trend is seen for all the 10 random primers used in this study. The data was used for similarit y coefficient calculation (Table 5).

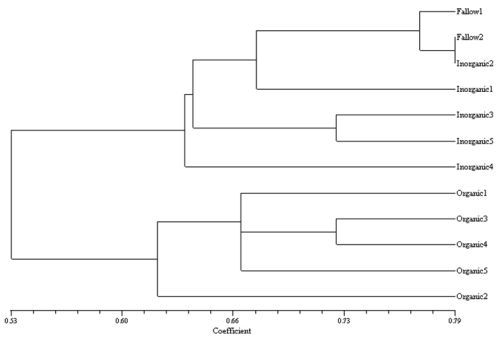
TABLE 5

Paired group Jaccard's coefficient matrix generated using RAPD data on soil DNA *Soil Biodiversity Analysis*

	F1	F2	I1	I2	I3	I4	I5	O1	O2	O3	O4	O5
F1	1.000											
F2	0.764	1.000										
I1	0.653	0.694	1.000									
I2	0.778	0.792	0.781	1.000								
I3	0.639	0.625	0.597	0.750	1.000							
I4	0.625	0.667	0.656	0.681	0.655	1.000						
I5	0.528	0.653	0.781	0.739	0.752	0.653	1.000					
O1	0.514	0.472	0.611	0.542	0.569	0.583	0.681	1.000				
O2	0.528	0.542	0.569	0.556	0.472	0.486	0.528	0.486	1.000			
O3	0.472	0.514	0.569	0.500	0.500	0.542	0.583	0.525	0.556	1.000		
O4	0.417	0.458	0.542	0.472	0.528	0.486	0.583	0.608	0.511	0.622	1.000	
O5	0.542	0.528	0.528	0.569	0.514	0.556	0.597	0.607	0.603	0.553	0.541	1.000

F1 and F2 refer to soil DNA obtained from fallow land and I1,I2,I3,I4, and I5 refer to soil DNA collected from inorganic fertiliser treated soil and O1,O2,O3,O4 and O5 refer to soil DNA obtained from organic manure treated farms respectively

The RAPD profile of all 10 random primers was analysed and the fragments were scored. The genetic similarity based on RAPD patterns in the form of Jaccard's similarity coefficient is presented in Table 5. Lesser values of similarity index were observed for organic soil samples and the average similarity within the organic soil samples was 0.577 (Table 5). In contrast, the inorganic soil samples had higher values and the average was 0.703 (Table 5). A dendrogram was constructed from Jaccard's average similarity coefficient of soils obtained in UPGMA analysis using RAPD data of 10 random primers (Figure 5). Cluster analysis indicates the inorganic fertiliser treated soils have low biodiversity equivalent to fallow soil seen as one cluster. The organic soil samples tend to cluster separately (Figure 5).



Fallow 1 and 2 refer to soil samples obtained from fallow land and Inorganic 1-5 and Organic 1-5 refer to soil samples collected from inorganic fertilizer treated and organic manure treated farms respectively

Figure 5: Dendrogram showing the grouping of soil samples based on soil microbial diversity observed from RAPD banding pattern.

DISCUSSION

Effect of organic fertilisers on soil quality was evaluated by comparing soil fertility status, microbial diversity and microbial dynamics in soils treated with organic manure vs inorganic fertilisers and chemicals. Nutrient status of two fallow soil samples was also compared. The organic manure treated soils had higher total carbon, nitrogen, phosphorus and potassium than the chemical fertilisers applied soils and fallow soil. A similar increase in total N, available P and exchangeable K content was observed in soils applied with organic manures (Adeniyan *et al*., 2011; Malero *et al.*, 2008). We also found that in the loamy soils of Coimbatore, organic fertilisation led to a smaller increase in N content (7%) compared to the increase observed in other nutrients such as C, P and K (59%, 52% and 108 %, respectively). Previously, Rosen and Allan (2007) had reported that lack of available nitrogen, synchronous with plant demand in organic cropping system, limits yields. Hence, for an increased yield, compost plus supplemental synthetic N applications is suggested (Valenzuela and Crosby, 1998)

Soil enzyme activity is the indicator for microbial dynamics. Activity of soil enzymes such as dehydrogenase, β-glucosidase, phosphatase and nitrate reductase was compared between organically fertilised and inorganic fertiliser treated soils. Higher enzyme activity was observed in organic manure applied soil samples than in inorganic fertilizer treated soil samples (Figures 1- 4). In the same way compost application in soil was found to increase the microbial biomass, respiration and dehydrogenase activity (Carpenter-Boggs *et al.*, 2000). Adding organic manure compost significantly increases the amount of cultivable microorganisms and microbial biomass, thus enhancing soil respiration and enzyme activities (Zhen *et al*., 2014). Increased enzyme activities of C and N (β-glucosidase, α-galactosidase, β-glucosaminidase,) and similar enzyme activities of P and S (alkaline phosphatase and arylsulfatase), were observed in a study comparing different poultry litter applications to cultivated Vertisols and pasture (Acosta-Martinez and Daren-Harmel, 2006). Among chemical fertiliser treated soil samples, I2 and organic manure treated soil samples, O2 showed minimum activity for all the enzymes (Figures 1- 4). This may be due to the soil type (Table 1). Samples treatment I2 and O2 are light, black cotton soil from Indore. The fertility status of light cotton growing soils is low as they are found to require more N and K application than heavier cotton growing soils of Virginia (Faircloth, 2007). This study also suggests that low fertility status might lead to a lower microbial activity in light soils in comparison with heavy cotton soils. We also observe that the heavy black cotton soils from both Indore and Coimbatore (Table 1) showed higher microbial activity (Figures 1- 4). Therefore, soil type seems to be a key determinant for microbial activity as suggested earlier by Girvan *et al.,* (2003).

Soil DNA content can be correlated to microbial biomass as all other debris were removed prior to DNA isolation. Extended polar lipid analyses such as phospholipids fatty acids (PLFA) and phospholipid ether lipids (PLEL) have also been performed to assess the microbial biomass (Esperschutz *et al.*, 2007). Light, black cotton soil from Indore showed a DNA content difference of 29% between I2 and O2 samples. However, the nutrient status and microbial activity in these samples were observed to be lower than most other soil samples. Hence DNA content may not be a direct indicator of microbial load and diversity as suggested earlier by Yang *et al*., (2000). They also had reported that chemical fertilisers cause an increase in soil biomass but a decrease in soil diversity by performing a RAPD analyis on soil DNA. They suggest that it could be due to chemical pollution, mainly ammonium bicarbonates and its intermediates.

Microbial diversity using RAPD analysis indicates a higher number of polymorphic fragments in organic soil than in inorganic and fallow soil (Figure 5) suggesting higher diversity. The diversity analysis indicates that microbial diversity in inorganic fertilizer treated soil (I2) is very minimal, equivalent to fallow soils as they group together (Figure 6). The microbial community is similar in all organic soil samples as they cluster together (Figure 6). Our findings imply that organic system of cropping favours microbial diversity and dynamics.

CONCLUSION

The present comparative analysis was performed on soils collected from organic and inorganic fertiliser treated soils. Soil fertility status is found to be correlated to soil type. Microbial diversity is found to be similar amongst the various organic manure treated soil samples as their DNA amplification profile is similar and they cluster together. The chemical fertilisers have an impact on the microbial population in the soil as seen by the reduced number of DNA bands and their clustering pattern. Results clearly indicate a significantly high nutrient status, microbial biomass, microbial diversity and microbial activity in organic manure treated soils compared to chemical fertiliser treated soils.

ACKNOWLEDGMENTS

The authors thank the management of PSG College of Technology Coimbatore for providing the infrastructure needed for the conduct of the experiments. They also thank Dr. Parvin Pandya of Jayvin Sales Private Limited, Indore for providing soil samples from Indore.

REFERENCES

Acosta Martinez, V., and Harmel, R.D., 2006. Soil microbial communities and enzyme activities under various poultry litter application rates. J. *Environ. Quality*. 35:1309-1318.

Adeniyan O. N., Ojo A. O., Akinbode, O. A. and Adediran J. A., 2011. Comparative study of different organic manures and NPK fertilizer for improvement of soil chemical properties and dry matter yield of maize in two different soils *J. Soil Sci. Environ. Manage.* 2: 9-13.

Barber, S.A. 1995 Soil nutrient bioavailability: A Mechanistic Approach, 2nd edn. Wiley, New York.

Carpenter-Boggs, L., Kennedy, A.C. and Reganold, J.P., 2000. Organic and biodynamic management: effects on soil biology. *Soil Sci. Soc. Am. J*. 64: 1651–1659.

Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Methods for Assessing Soil Quality, Soil Sci. Soc. Am. Madison, WI 9: 9-17.

Esperschütz J., Gattinger A., Mäder P., Schloter M., and Fließbach A., 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. *FEMS Microbiol.* Ecol. 61: 26-37.

Faircloth, J. (ed). 2007. Cotton Fertility, Virginia Cotton Production Guide. PP. 1-2

Fauci, M.F. and Dick, R.P. 1994. Microbial Biomass as an Indicator of Soil Quality: Effects of Long-Term Management and Recent Soil Amendments. In: Defining Soil Quality for a Sustainable Environment, Doran, J.W. and A.J. Jones (Eds.). SSSA, Madison, WI., pp: 229-234.

Girvan, M. S., J. Bullimore, J. N. Pretty, A. M. Osborn, and Ball, A.S. 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl. Environ. Microbiol.* 69:1800-1809.

Hooda S, Kaur S. 1999; Laboratory Manual for Environmental Chemistry. 1st. ed. S. Chand & Company Ltd.: New Delhi, pp80-85.

Indian fertilizer scenario- 2014. http://fert.nic.in/sites/default/files/Indian%20Fertilizer%20SCENARIO-2014_0.pdf

Krishnakumar, S., Saravanan, A. Natarajan, S.K. Veerabadran, V., and S. Mani, S., 2005. Microbial Population and Enzymatic Activity as Influenced by Organic Farming. *Res. J. Agr and Biol. Sci.* 1: 85-88.

Latour A., Corberand T., Laguerre G., Allard F., and Lemanceau. P., 1999. The composition of fluorescent Pseudomonas population associated with roots is influenced by plant and soil type, *Appl. Environ. Microbiol*. 62: 2449–2456.

Latour, A., Corberand, T.S., Laguerre, G., Allard, F., and Lemanceau, P., 1996. The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. *Appl Environ Microbiol*. 62: 2449–2456.

Madejón, E., Moreno, F., Murillo, J.M., and Pelegrín, F., 2007. Soil biochemical response to long-term conservation tillage under semi-arid Mediterranean conditions. *Soil and Tillage Research*. 94: 346-352.

Mäder P., Fliessbach A., Dubois D., Gunst L., Fried P., and Niggli U., 2002. Soil fertility and biodiversity in organic farming. *Science*. 296: 1694-1697.

Martina S. Girvan, M.S. Bullimore, J. Pretty, J.N Osborn, A.M. and Ball A.S., 2003. Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. *Appl. Environ. Microbiol*. 69: 1800–1809.

Malero, S. Madejón, E. Herencia, J.F. and Ruiz, J.C. 2008. Effect of implementing organic farming on chemical and biochemical properties of irrigated loam soil. *Agronomy J*. 100: 136-144.

Nimisha S, Sudarsan, Y., Sharma, R. and Singh, G., 2008. RAPD analysis of soil microbial diversity in western Rajasthan. *Current science*. 94(8), pp.1058-1061.

Oberholtzer, L., C. Dimitri, and C.Greene. 2005. Price premiums hold on as U.S. organic produce market expands. USDA Economic Res. Serv. VGS-308- 01.

Omay, A.B., Rice, C.W., Maddux, L.D., Gordon, W.B., 1997. Changes in soil microbial and chemical properties under long-term crop rotation and fertilization. *Soil Sci. Soc. Am. J.* 61: 1672–78.

Pape, A.L. Miller, R.H., Keeny, D.R. (eds) 1982. Methods in Soil Analysis, part2; Chemical and Microbiological Properties. Soil Sci. Soc.Am. Am. Soc.Agron. SSSA Book, Madison WI

Parham, J.A., Deng, S.P., Raun, W.R., Johnson, G.V., 2002, Long-term cattle manure application in soil. I. Effect on soil phosphorus levels, microbial biomass C, and dehydrogenase and phosphatase activities. *Biol. Fertil. Soils* 35: 328–337.

Pepper, I.L., Gerba, C.P., Brendecke, J.W., 1995. Dehydrogenase Activity of Soils. Environmental Microbiology: A Lab Manual, pp 51-56. (Academic Press, San Diego, CA, USA).

Robertson, G. P., and S. M. Swinton. 2005. Reconciling agricultural productivity and environmental integrity: A grand challenge for agriculture. *Frontiers in Ecology and the Environment.* 3: 38-46.

Rosen, C.J. and Allan, D.L., 2007. Exploring the benefits of organic nutrient sources for crop production and soil quality. *Hort Technology*.17: 422 - 30.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Plainview, NY.

Tabatabai, M.A. 1994. Soil enzymes. In: Weaver,R.W., J.S. Angle, and P.S. Bottomley, (eds). Methods of Soil Analysis. Part II: Microbiological and Biochemical Properties. Soil Sci. Soc. Amer., Madison, WI. 775-833.

Valenzuela, H.R., and C. Crosby,C. 1998. Effect of compost applications on the yield of several vegetables in long-term organic farming experiments conducted in the tropics. *Hort. Sci.* 33: 447-524.

Yang, Y. Yao, J. Hu, S. and Qi, Y. 2000. Effects of agricultural chemicals on DNA sequence diversity of soil microbial community: A study with RAPD Marker. *Microb. Ecol*. 39:72-79.

Zhen, Z. Liu, H. Wang, N. Guo, L. Meng, J. Ding, N. Wu, G. and Jiang, G.2014. Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. *PLoS ONE* 9(10):