SOIL MICROBIAL POPULATION AND ENZYME ACTIVITIES UNDER ORGANIC, BIODYNAMIC AND CONVENTIONAL AGRICULTURE IN SEMI-ARID TROPICAL CONDITIONS OF CENTRAL INDIA

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KEYWORDS
Alkaline phosphatase
Biodynamic
Dehydrogenase
Fluorescein diacetate
Fungi
Microbial biomass
Organic agriculture

ABSTRACT

Present field experiment was conducted at the Indian Institute of Soil Science, Bhopal, India in a clayey soil (Typic Haplusterts) under soybean (Glycine max, cv. JS 335) wheat (Triticum durum, cv. HI 8498) cropping system in a randomized block design with seven treatments in four replications to study the changes in soil fungal, bacterial and actinomycetes population; and resultant enzymatic activities in soil under organic, biodynamic and conventional agriculture management. The results of study revealed that, the soil microbial population (bacteria, fungi and actinomycetes), soil enzyme activities and soil microbial biomass carbon were found in the order of organic > conventional ≥ biodynamic agriculture. The organic agriculture registered 27-110% and 28-111% higher enzymatic activities than conventional and biodynamic agriculture, respectively. Similarly, soil microbial biomass carbon was found 30-45% and 33-42% higher under organic agriculture management as compared to conventional and biodynamic agriculture management, respectively. No significant effect of biodynamic agriculture management on soil microbial properties was observed.
1 Introduction

Chemical fertilizers play an important role to meet nutrient requirement of the crop and their increased use has been an important tool in the drive for increased crop production (Fan, 1991). In the present scenario of energy crisis, the cost of fertilizers is high and inadequate (Dawson & Hilton, 2011). Also, increase in production and consumption of mineral fertilizers seriously affects the natural environment through air, water and soil pollution (Tilman et al., 2002). The continuous and large/imbalanced quantities of mineral fertilizers have slowed down and in some cases there are indications of decline in soil productivity and production (Reganold et al., 1987; Foley et al., 2005). Reduced agricultural productivity, deteriorated soil health, escalating production costs, heavy reliance on non-renewable resources, depleting soil organic carbon, reduced microbial diversity (Zhong & Cai, 2007), water contamination, chemical residues in food grains and health risk to the population are the main reason to think for substituting the nutrient requirement of the crops through organic inputs (Carvalho, 2006). Recent studies have highlighted the substantial contribution of organic farming towards protection and conservation of environment and food safety, quality and security (Scialabba & Hattam, 2002; Aher et al., 2015).

Considering the above facts present study was conducted to evaluate the changes in soil microbial population (fungi, bacteria and actinomycetes), soil enzyme activities viz., dehydrogenase, alkaline phosphatase and fluorescein diacetate; and soil microbial biomass carbon under organic, conventional and biodynamic agriculture management practices in a Vertisol soil of Central India.

2 Materials and methods

2.1 Study Site and Climate

Present investigation was conducted under Network Project on Organic Farming (NPOF) at Research Farm of the Indian Institute of Soil Science, Bhopal. Geographically, the experimental site lies between 23°18’ N latitude and 77°24’ E longitudes. The elevation above mean sea level is 495 m. The site falls under sub-humid tropics with an average annual rainfall of 1208 mm and a mean annual air temperature of 25°C. Out of total annual rainfall, about 80 per cent is received from South-West monsoon (June to September), while the rest from North-East monsoon (October and November). Agro-ecologically, the study region lies in Vindhya plateau region (Zone No. IV) having sub-humid climate.

2.2 Characteristics of experimental field

The soil of experimental site is classified as Vertisol (Typic Haplusterts) with smectite as the dominant clay mineral. Vertisols are churning heavy clay soils with a high proportion of swelling clays. These soils form deep wide cracks from the surface downward when they dry out, which happens in most years. The initial characteristics of the experimental soil are presented in Table 1. The soil of the experimental site is clayey in texture (Typic Haplusterts) with 25.2, 18 and 56.8 per cent of sand, silt and clay, respectively. Initially, the soil (0 to 15 cm depth) was medium in soil organic carbon (0.53%), low in available N (68.8 mg kg\(^{-1}\)), medium in available P (12.8 mg kg\(^{-1}\)) and high in available K (237 mg kg\(^{-1}\)). The soil was slight alkaline in reaction (pH 7.76) with 0.48 dS m\(^{-1}\) electrical conductivity.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>25.2 ± 2.3</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>18.0 ± 1.8</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>56.8 ± 4.2</td>
</tr>
<tr>
<td>pH(1:2)</td>
<td>7.76 ± 0.03</td>
</tr>
<tr>
<td>EC(1:2) dS m(^{-1})</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Available N (mg kg(^{-1}))</td>
<td>68.8 ± 9.8</td>
</tr>
<tr>
<td>Available P (mg kg(^{-1}))</td>
<td>12.8 ± 0.8</td>
</tr>
<tr>
<td>Available K (mg kg(^{-1}))</td>
<td>237 ± 12.2</td>
</tr>
</tbody>
</table>

2.3 Field Experiment and Treatments

The experiment was conducted in randomized block design with seven treatments in four replications. The experiment consisted of OM-Organic manure on nitrogen equivalent basis, BD-Biodynamic Preparation (BD 500 (Cow Horn Manure) as soil application @ 75 g ha\(^{-1}\) + BD 501 (Cow Horn Silica) as foliar application @ 2.5 g ha\(^{-1}\)), OM+PG- Organic Manure on nitrogen equivalent basis + foliar spray of 3% Panchagavya, OM+BD- Organic Manure on nitrogen equivalent basis+ Biodynamic Preparation (BD 500 - Cow Horn Manure) as soil application @ 75 g ha\(^{-1}\) + BD 501 (Cow Horn Silica) as foliar application @ 2.5 g ha\(^{-1}\)), OM+PG+BD- Organic Manure on nitrogen equivalent basis+ Biodynamic Preparation (BD 500(Cow Horn Manure) as soil application @ 75 g ha\(^{-1}\) + BD 501 (Cow Horn Silica) as foliar application @ 2.5 g ha\(^{-1}\))+ foliar spray of 3% Panchagavya,
Control and RDF - Recommended dose of chemical fertilizers N:P:K through urea, single super phosphate and murate of potash, respectively (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>Organic Manure*</td>
</tr>
<tr>
<td>BD</td>
<td>Biodynamic Preparations</td>
</tr>
<tr>
<td>OM + PG</td>
<td>Organic Manure + Panchagavya</td>
</tr>
<tr>
<td>OM + BD</td>
<td>Organic Manure + Biodynamic Preparations</td>
</tr>
<tr>
<td>OM + PG + BD</td>
<td>Organic Manure + Biodynamic Preparations + Panchagavya</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>RDF</td>
<td>Recommended Dose of Chemical Fertilizers</td>
</tr>
</tbody>
</table>

*For soybean crop the organic manure (OM) is cattle dung manure (CDM) and for wheat crop organic manure is cattle dung manure (CDM) + vermicompost (VC) + poultry manure (PM) in 1:1:1 proportion on nitrogen equivalent basis.

Cattle dung manure (CDM), vermicompost (VC) and poultry manure (PM) were used as organic manure and was applied on the N equivalent basis with due adjustment of moisture in all the treatments involving application of organic manure. The panchagavya was prepared by mixing of cow dung, cow urine, milk, curd and ghee in 3:2:2:1 proportion in 3 liter of water and incubated for 10 days in a wide mouth plastic container. In addition to five products, jaggery (500 g), ripened banana fruit (12 nos.) and water of tender coconut (3 liter) were also added to improve the fermentation process. The contents were stirred daily clockwise and anticlockwise during morning and evening. The prepared panchagavya were filtered with cloth and used for foliar spray (3%). The biodynamic preparation BD 500 (Cow Horn Manure) and BD 501 (Cow Horn Silica) were commercially procured from Kurinji Organic Food (I) Pvt. Ltd., Theni - 625203, Tamil Nadu, India. The mean nutrient content of the organic inputs used in this experiment was cattle dung manure (0.86% N), P (0.44%); vermicompost (1.09% N), P (0.73%) and K (0.82%); poultry manure N (1.63%), P (1.17%) and K (1.29%); panchagavya N (0.78%), P (0.19%) and K (0.35%); Cow horn manure (BD 500) N (2.12%), P (0.81%) and K (0.82%) whereas in cow horn silica (BD 501) no nutritional element was detected (Table 3). Cattle dung manure and cow horn manure (BD 500) was supplied as basal application before last plough. Doses of fertilizers in recommended dose of chemical fertilizer treatment were given as basal application at the time of sowing. The panchagavya (3%) and cow horn silica (BD 501) were used as foliar spray and soil application.

Disease and pest free seeds of soybean (cv. JS-335) and wheat (cv. HB498) were used for sowing with 45 X 5 cm and 22.5 X 5 cm spacing, respectively. The seed was treated with phosphate solubilizing micro-organisms (PSB) and Rhizobium having $10^9$ viable cell g$^{-1}$ (Manufactured by Biofertilizer Unit of Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur) @ 20 g kg$^{-1}$ seed. The crop soybean was raised under rainfed conditions while 4 irrigations were applied to wheat crop. Neem Oil (Azadiractin 3%; Make: Pest Control (I) Pvt. Ltd) and Hostathion (Trizophos 40 EC; Make: Bayer Crop Science Ltd.; @ 0.75 lit ha$^{-1}$) was used to control the pests in organic and chemical fertilizer treatments, respectively. Pheromone Traps were also used in organic plots in order to control the crop specific pest Helicoverpa armigera and Spodoptera litura of soybean. The yield of each crop was recorded at harvest on the basis of total weight of seed and grain of soybean and wheat, respectively from the experimental plot. The soybean in kharif and wheat in rabi (2011-12 and 2012-13) were grown with the selected set of treatments. In organic treatments, nutrients were applied through cattle dung manure to the soybean crop during the rainy season (July-October) and a combination of cattle dung manure + vermicompost + poultry manure (one third each) to wheat during the winter season (November-March) (Table 4). These manures were applied on the N equivalent basis to with due adjustment of moisture in manure (Table 3 and Table 4). The nutrients in the RDF treatment plots were supplied through chemical fertilizers.

### 2.4 Analysis of organic manures for N, P and K

The macronutrient composition of applied manures was determined before field application. The collected sample of manures was initially air dried and further kept in oven at 65°C till constant weight was reached. These samples were powdered in grinder and used for determining concentration of total N, P and K.

<table>
<thead>
<tr>
<th>Nutrient (g kg$^{-1}$)</th>
<th>CDM</th>
<th>VC</th>
<th>PM</th>
<th>PG</th>
<th>BD 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>8.6 ± 0.63</td>
<td>10.9 ± 1.79</td>
<td>16.3 ± 0.75</td>
<td>7.8 ± 0.11</td>
<td>21.1 ± 1.29</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>4.4 ± 0.11</td>
<td>7.3 ± 0.85</td>
<td>11.7 ± 0.25</td>
<td>1.9 ± 0.05</td>
<td>8.1 ± 0.16</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>10.7 ± 0.74</td>
<td>8.2 ± 0.60</td>
<td>12.9 ± 0.31</td>
<td>3.5 ± 0.15</td>
<td>8.2 ± 0.03</td>
</tr>
</tbody>
</table>

CDM- cattle dung manure; VC- vermicompost; PM- poultry manure; PG- panchagavya; BD 500- biodynamic preparation (cow horn manure). In BD 501 no nutritional element was detected.
K. Total nitrogen in manures was determined in microkjeldahl after digesting in concentrated sulphuric acid (Parkinsons & Allen, 1975; Bremner & Mulvaney, 1982). For determination of P and K the samples were digested in a mixture of HNO₃ and HClO₄ (9:4) as suggested by Singh et al. (2005). The total P in digest was determined by Vanadomolybdate yellow colour method as outlined by Jackson (1973) whereas total K was determined using flame photometer (Chapman & Pratt, 1961).

### 2.5 Soil sampling, processing and analysis

The soil samples were collected during both the years of study at the end of soybean-wheat crop cycle from 0-15 cm depth at three randomly selected spots in each replication and composite samples were prepared. The samples were air dried, gently ground, well mixed and sieved through 2 mm mesh and utilized for laboratory analysis for chemical and biological properties. For microbial analysis the field moist soil samples were collected and stored at 4°C until further analysis.

Soil chemical parameters were determined by following standard methods (Jackson, 1973). The soil pH and EC were determined (1:2 soil:water suspension) on potentiometer. The available N (Subbiah & Asija, 1956); available P (Olsen et al., 1954); exchangeable K (Hanway & Heidel, 1952) and soil organic C (Walkley & Black, 1934) was estimated from soil samples. The labile carbon (potassium permanganate easily oxidizable C) in soil was determined following the procedure of Blair et al. (1995) and Weil et al. (2003). Soil microbial biomass carbon from soil was determined by the fumigation-extraction method described by Jenkinson & Powlson (1976) and later modified by Vance et al. (1987). The soil microbial enzyme activities viz., alkaline phosphatase (Tabatabai & Bremer, 1969), soil dehydrogenase activity (Casida et al., 1964) and soil fluorescein diacetate enzyme activity (Schnurer & Rosswall, 1982) were measured by following the standard methods. Soil microbial population viz., bacteria, fungi and actinomycetes in soil samples were determined by adopting the method suggested by Halvorson & Ziegler (1933). The microbial population of bacteria, fungi and actinomycetes were expressed as colonies forming unit per gram soil (cfu g⁻¹ soil). The cfu g⁻¹ of soil bacteria, fungi and actinomycetes, were determined by using the serial dilution pour plate technique using their respective media (Table 5).

### Table 4 Experimental details of soybean-wheat sequence

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Soybean</th>
<th>Test crops</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variety</strong></td>
<td>JS-335</td>
<td>HI 8498</td>
</tr>
<tr>
<td><strong>Nutrient (N:P:K) dose (kg ha⁻¹)</strong></td>
<td>30:26:2:16.6</td>
<td>80:17.5:33.2</td>
</tr>
<tr>
<td><strong>Design</strong></td>
<td>RBD</td>
<td>RBD</td>
</tr>
<tr>
<td><strong>Replications</strong></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Plot size (m²)</strong></td>
<td>6 x 7</td>
<td>6 x 7</td>
</tr>
<tr>
<td><strong>Seed rate (kg ha⁻¹)</strong></td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td><strong>Spacing (cm x cm)</strong></td>
<td>45 x 5</td>
<td>22.5 x 5</td>
</tr>
</tbody>
</table>

### Table 5 Composition of media used for growing actinomycetes, fungi and bacteria

<table>
<thead>
<tr>
<th>Kenknight’s agar medium (Actinomycetes)</th>
<th>Nutrient agar medium (Bacteria)</th>
<th>Potato dextrose agar medium (Fungi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 1.0 g</td>
<td>Beef extract 3 g</td>
<td>Potato (Peeled) 250 g</td>
</tr>
<tr>
<td>Monopotassium phosphate 0.1 g</td>
<td>Peptone 5 g</td>
<td>Dextrose 20 g</td>
</tr>
<tr>
<td>Potassium chloride 0.1 g</td>
<td>Agar 20 g</td>
<td>Agar 20 g</td>
</tr>
<tr>
<td>Magnesium sulphate 0.1 g</td>
<td>Sucrose 20 g</td>
<td>Distilled water 1000 ml</td>
</tr>
<tr>
<td>Agar 15.0 g</td>
<td>Distilled water 1000 ml</td>
<td>pH 6.0-6.5</td>
</tr>
<tr>
<td>Sodium nitrate 0.1 g</td>
<td>pH 6.8-7.2</td>
<td></td>
</tr>
<tr>
<td>Distilled water 1000 ml</td>
<td>pH 7.0-7.2</td>
<td></td>
</tr>
</tbody>
</table>
Table 6 Effect of organic farming practice on soil organic carbon and its fractions in 0-15 cm soil depth under soybean-wheat cropping system (Pooled data of 2011-12 and 2012-13)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>EC</th>
<th>SOC</th>
<th>KMnO₄-C</th>
<th>SMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>7.84&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.62&lt;sup&gt;+&lt;/sup&gt;</td>
<td>551&lt;sup&gt;+&lt;/sup&gt;</td>
<td>274&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>BD</td>
<td>8.01&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.29&lt;sup&gt;+&lt;/sup&gt;</td>
<td>309&lt;sup&gt;b&lt;/sup&gt;</td>
<td>203&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>OM + PG</td>
<td>7.94&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.82&lt;sup&gt;+&lt;/sup&gt;</td>
<td>533&lt;sup&gt;b&lt;/sup&gt;</td>
<td>288&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>OM + BD</td>
<td>7.92&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.66&lt;sup&gt;+&lt;/sup&gt;</td>
<td>523&lt;sup&gt;c&lt;/sup&gt;</td>
<td>270&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>OM + PG + BD</td>
<td>7.92&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;+&lt;/sup&gt;</td>
<td>7.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>539&lt;sup&gt;b&lt;/sup&gt;</td>
<td>279&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>8.02&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.34&lt;sup&gt;+&lt;/sup&gt;</td>
<td>304&lt;sup&gt;c&lt;/sup&gt;</td>
<td>200&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>RDF</td>
<td>7.96&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6.74&lt;sup&gt;+&lt;/sup&gt;</td>
<td>319&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

EC- electrical conductivity (dS cm⁻¹); SOC- soil organic carbon (g kg⁻¹), KMnO₄-C- potassium permanganate oxidizable carbon (mg kg⁻¹) and SMBC- soil microbial biomass carbon (mg kg⁻¹); Columns with different lower case letters are significant according to Duncan’s Multiple Range Test (P<0.05)

### 2.6 Statistical analyses

The primary data generated through observations and laboratory analysis during the investigation was compiled, pooled, statistically analyzed and the differences among the treatment means were tested for their significance (P<0.05) as outlined by Gomez & Gomez (1984) using Web Agri Stat Package 2.0 (WASP 2.0) developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India.

### 3 Results

#### 3.1 Soil pH and electrical conductivity (EC)

The pH of the soil was found neutral to slightly alkaline in nature across all the treatments (Table 6). It has been observed that the organic farming practice or application of chemical fertilizers had no marked difference on soil pH over the brief period of study which may be attributed to the fact that the soil pH is mainly affected by the parent material involved in soil formation and the climatic conditions. The electrical conductivity of soil ranged from 0.20 dS cm⁻¹ to 0.29 dS cm⁻¹ with highest value in treatment receiving recommended dose of chemical fertilizers (RDF). Significant decrease in electrical conductivity of OM, OM+PG, OM+BD and OM+PG+BD over other treatments was observed while these treatments were statistically at par with each other. Treatment RDF was statistically significant over all other treatments whereas BD and Control were found statistically at par. It has been observed that the treatments receiving organic manure alone or in combination showed slight but significant reduction in electrical conductivity (Table 6).

#### 3.2 Soil organic carbon (SOC)

The SOC in different treatment combinations ranged from 6.29 g kg⁻¹ to 7.82 g kg⁻¹ with highest in treatment OM+PG followed by OM+PG+BD (7.73 g kg⁻¹). The lowest value was recorded in treatment BD. The treatment RDF was found statistically superior over BD and Control. The treatments receiving organic manure along with panchagavya (OM+PG and OM+PG+BD) and the treatments OM and OM+BD recorded significantly higher SOC than any other treatment. These treatments were found statistically superior over RDF (6.74 g kg⁻¹), BD (6.29 g kg⁻¹) and Control (6.34 g kg⁻¹). The treatments receiving organic manures either alone or in combinations reported 13-16% increases in SOC over the treatment receiving recommended dose of chemical fertilizers (RDF) (Table 6).

#### 3.3 Potassium permanganate oxidizable carbon (Labile C)

The KMnO₄-C (Labile C) was ranged from 304 mg kg⁻¹ in Control to 551 mg kg⁻¹ in OM followed by OM+PG+BD (539 mg kg⁻¹), OM+PG (533 mg kg⁻¹) and OM+BD (523 mg kg⁻¹). The treatments receiving organic manures alone or in combination were found statistically at par with each other but found significant over Control and BD including RDF. The organic treatments viz. OM+PG+BD, OM+PG, OM and OM+BD increased KMnO₄-C by 64-73% over RDF. No significant difference in labile C was found between the treatments BD, Control and RDF (Table 6).

#### 3.4 Soil microbial biomass carbon (SMBC)

The SMBC ranged from 200 mg kg⁻¹ in control to 288 mg kg⁻¹ in OM + PG (Table 6). The treatment receiving biodynamic preparations (BD) was found statistically at par with Control. The treatments involving organic soil amendments i.e. OM+PG+BD, OM+PG, OM and OM+BD were noticed for significantly higher SMBC than RDF, BD and Control but were found statistically at par with each other. The treatment RDF was found statistically significant over BD and Control. The treatments involving
applications of organic soil amendment either alone or in combinations increased SMBC by 35-44% and 15-22% over Control and RDF, respectively.

3.5 Soil microbial population

The microbial population of bacteria, fungi and actinomycetes during the two years ranged between 4.0x10^7 - 12.5x10^7 cfu g^-1, 3.5x10^6 - 8.5x10^6 cfu g^-1 and 2.6x10^6 - 7.5x10^6 cfu g^-1 soil, respectively (Table 7). The bacterial population was found to be the highest in treatment OM+PG+BD followed by OM+PG (12.0x10^7 cfu g^-1), OM (10.8x10^7 cfu g^-1) and OM+BD (10.8x10^7 cfu g^-1). These treatments were found statistically superior over BD (4.4x10^7 cfu g^-1), RDF (4.3x10^7 cfu g^-1) and Control (4.0x10^7 cfu g^-1). The results revealed that the treatments OM+PG+BD, OM+PG, OM and OM+BD; and treatments BD, Control and RDF were statistically at par. Similar trends to that of bacterial population were observed for the population of fungi and actinomycetes in all the treatment combinations. The data in Table 7 indicated higher soil microbial population in treatments receiving organic nutrient inputs as compared to other treatments.

3.6 Soil enzyme activities

The soil enzyme activities viz. fluorescein diacetate (FDA), dehydrogenase (DHA) and alkaline phosphatase (Alk-PO₄) are presented in Table 7. The FDA activity varied from 25.3 to 56.6 µg fluorescein g^-1 h^-1 with highest in treatment OM+PG+BD. The FDA activity in treatments OM+PG+BD, OM+PG, OM and OM+BD were found statistically significant over other treatments viz. BD (26.8 µg fluorescein g^-1), Control (25.3 µg fluorescein g^-1) and RDF (28.0 µg fluorescein g^-1). The treatments OM+PG+BD, OM+PG, OM and OM+BD and treatments BD, Control and RDF were found statistically at par (Table 7).

The DHA activity ranged between 61.0 and 110.6 µg TPF g^-1 day^-1 with lowest and highest values were observed for treatment Control and OM+PG+BD, respectively. The DHA activity in treatments OM+PG+BD, OM+PG, OM and OM+BD were significantly higher than the treatments BD, Control and RDF. The treatments OM+PG+BD, OM+PG, OM and OM+BD; and the treatments BD, Control and RDF were found statistically at par. Like FDA and DHA activities, Alk-PO₄ activity in soil also followed similar trends across the treatments. The values ranged from 105-149.8 µg PNP g^-1 h^-1 with higher value in treatment OM+PG followed by OM+PG+BD (146.2 µg PNP g^-1 h^-1) (Table 7). With respect to the Alk-PO₄ enzyme activity the treatments were found in the order of OM+PG = OM+PG+BD = OM = OM+BD > RDF = BD = Control. No significant changes in the enzyme activity in treatment BD were noticed during either years of study. It has been observed that, the treatments receiving organic manure alone or in combinations reported significantly higher soil enzyme activities (Table 7).

4 Discussion

4.1 Soil organic carbon, labile carbon and microbial biomass

The soil organic carbon (SOC) showed highest accumulation under the treatments receiving organic manures alone or in combination with liquid organics. Application of OM along with liquid organics resulted 20-23% increase in SOC over control and 13-16% over conventional farming (Table 6). The observed increase in SOC might be due to the continuous buildup of carbon in soil under organic practice as it relies on external carbon inputs such as cattle dung manure, vermicompost and poultry manure. Besides the regular applications of different organic manures, the root biomass and left over stubbles have also contributed to the increment in carbon pools. Jha et al. (2014) observed an increment of 49.1% in total SOC with the application of FYM @ 15 t ha^-1 Y^-1 along with recommended dose of NPK on long term basis (38 years) over control in a Vertisol of Jabalpur under soybean-wheat sequence. Similarly, Lakaria et al. (2012a) also reported 105 and
71% higher TOC in long term organic farming practice over absolute control and recommended dose of NPK fertilizers, respectively under soybean-wheat cropping system. Manna et al. (2012) and Lakaria et al. (2012b) also reported increase in SOC with the application of FYM alone or in combination with recommended NPK fertilizers over absolute control and sole NPK fertilizer application. The results of this study are in close agreement with these findings. The higher C accumulation in the Vertisol may be attributed to their high silt-clay content which increase the C stabilization capacity (Six et al., 2002, Jha et al., 2012a).

The potassium permanganate oxidizable carbon (KMnO₄-C) comprised of amino acids, simple carbohydrates, fraction of SMB and other carbon compound and represents the labile pool of SOC (Zou et al., 2005). Labile pool of carbon is the fraction of SOC that has the most rapid turnover rates (Verma et al., 2010) and therefore, its oxidation drives the flux of carbon dioxide from soils to atmosphere. Also, the labile carbon pool is one which is readily decomposable, easily oxidizable and susceptible to microbial attack and is sensitive to management induced changes in soil organic carbon. This pool is very important as it fuels the soil food web and greatly influences the nutrient cycling for maintaining the quality of soil and its productivity (Majumder et al., 2008). In this study, the soil KMnO₄-C content was ranged from 304 mg kg⁻¹ to 551 mg kg⁻¹. Lakaria et al. (2012b) and Jha et al. (2012b) also observed the KMnO₄-C content between 463 and 621 mg kg⁻¹ and 311.8 and 555.5 mg kg⁻¹ under soybean-wheat rotation in a Vertisol. Further, the results showed that the continuous application organic manures significantly improved soil KMnO₄-C content as compared to application of chemical fertilizers, sole biodynamic and absolute control. The organic treatments increased KMnO₄-C by 72-81% and 64-73% over Control and RDF, respectively. Lakaria et al. (2012b) and Jha et al. (2012b) also reported 12% and 34%; 12% and 99% higher KMnO₄-C under organic farming than conventional farming and control, respectively. The higher KMnO₄-C under the treatment receiving organic manures alone or in combination with liquid organics (panchagavya and/or biodynamic preparations) might be attributed to the continuous buildup of carbon in soil as present experiment on organic farming relies on external carbon inputs such as cattle dung manure, vermicompost and poultry manure. Besides the regular applications of different organic manures, the root biomass and left over stubbles have also contributed the possible increment. The increase in KMnO₄-C was resulted from the growth and decomposition of organic materials. Plant roots could break down the carbon compound into labile carbon (Conteh et al., 1997). Labile carbon functions as the source of energy for the growth of soil microbes that is important for development of soil microbial population and influence the rate of organic material decomposition. Sardiana et al. (2014) also reported that the KMnO₄-C under organic system was significantly higher (49.01%) than that in conventional farming system. Our results are in close agreement with these results.

The soil microbial biomass carbon (SMBC) is considered to be the most active and highly labile fractions of SOC and incorporation of organic manures and residues substantially improves the labile pool of carbon hence their assay provides the meaningful information about its accumulation and dynamics in soil. The SMBC is considered to be an important early indicator of changes that may occur in the long term with regard to soil fertility and constitutes an important source and sink of nutrients (Moussa et al., 2007). In present investigation, the soil microbial biomass carbon in 0-15 cm soil depth ranged between 200 and 288 mg kg⁻¹ under different treatment combinations, respectively (Table 6). Lakaria et al. (2012c) found that the SMBC was ranged from 113 mg kg⁻¹ to 430.7 mg kg⁻¹ under different land use whereas Jha et al. (2012b) recorded SMBC in the range of 88.9 to 430.7 mg kg⁻¹ under different treatments in Vertisols. Recently Lori et al. (2017) also reported 32-84% higher soil microbial biomass carbon under organic farming as compared to conventional farming practices. The results also showed that the SMBC was higher under the treatments receiving the organic manure either alone or in combinations with liquid manures (Table 6). The increase of SMBC in organic plots is, probably, due to the higher contents of more readily decomposable C fractions in the added organic manure (Rochette et al., 2006) which served as a food for microbial propagation and also due to the microbial biomass contained in the organic matter itself (Gattinger et al., 2004). The results are also in agreement with the findings of Melero et al. (2006), Tu et al. (2006), Araujo et al. (2009), Lakaria et al. (2012b), Jannoura et al. (2014) in soils under organic farming systems. According to Fliebach & Mader (2000), over the long term, SMBC is significantly affected by the long-term organic management as well as by its intensity.

4.2 Soil microbial population and enzyme activities

The data on the soil microbial population (bacteria, fungi and actinomycetes) has followed a trend of natural population i.e. predominance of bacteria followed by actinomycetes and fungi. However, there were significant variations in population of soil bacteria, actinomycetes and fungi at the end of cropping cycle among different treatments combinations. The data indicated higher soil microbial population in treatments receiving organic manures compared to recommended dose of fertilizers, biodynamic application and absolute control (Table 7). Patil & Varade (1998) observed that the application of FYM significantly enhanced the population of fungi, bacteria and actinomycetes. These results on microbial population in present study are in close agreement with the earlier observations reported by Graham &
Haynes (2005), Sanzano et al. (2009). The increased microbial population under organic manure application mainly attributed to the higher organic carbon especially biologically active phase of carbon which acted as source of energy for microbes proliferating in soil as reported by Rajannan & Oblisami (1979). Similarly, the significant positive correlation among soil organic carbon and microbial population has already been explored earlier (Dick et al., 1994, Graham & Haynes, 2005). Chang et al. (2007) also revealed that the soil microbial biomass, populations of bacteria, fungi and actinomycetes, increased significantly in compost treated soils over application of only chemical fertilizers and microbial activities showed significant linear correlations with the organic matter contents of the soils. The enhanced microbial population upon application of different sources of organic matter viz. FYM/CDM (Bulluck et al., 2002; Kannan et al., 2006), vermicompost (Parthasarathi et al., 2003), poultry manure (Nwangburuka et al., 2012), panchagavya (Xu, 2001), crop residues (Sanzano et al., 2009), biodynamics (Bougrom et al., 2012) are in close agreement with our results. Liu et al. (2017) reported significantly higher microbial population in organically managed plots as compared to the conventional practice.

Chemically enzymes are specialized proteins that act as biocatalyst either by accelerating or restricting rate of biochemical reactions without undergoing permanent change in original enzyme status. In soil, enzyme can exist intracellular (inside the cytoplasmic membranes of living cells) or extracellular (outside the cytoplasmic membrane) and involved in soil bio-geochemical processes such as organic matter decomposition, humus formation and nutrient cycling (Sinsabaugh et al., 1991). Dick et al. (1994) reported that, the activities of soil enzymes viz. alkaline phosphatase, dehydrogenase and fluorescein diacetate (FDA) are highly correlated to soil organic carbon which releases energy for micro-organisms and suggested that, their assay in soil provides a broad-spectrum indicator of soil biological health. In present study, the soil enzyme activities viz. fluorescein diacetate (FDA), dehydrogenase (DHA) and alkaline phosphatase (Alk-PO₄) were significantly influenced by organic farming practices. The FDA, DHA and Alk-PO₄ enzyme activities showed 89-102%, 62-72% and 27-35% increment under organic treatments as compared the treatment involving application of chemical fertilizers, respectively (Table 7). The increment might be attributed to the higher organic carbon content in these plots. The soil enzyme activities are positively significantly correlated with TOC, active, slow and passive pools of carbon, soil respiration, microbial biomass and soil available nitrogen (Casida et al., 1964; Juma & Tabatabai, 1977; Rajannan & Oblisami, 1979; Klein & Koh, 1980; Graham & Haynes, 2005). The organic farming practices are known to increase the microbial enzyme activities in soil as reported by Marinari et al. (2006) and Fliebach et al. (2007). Results of the present investigation are in accordance with other researchers who also observed higher enzyme activities with the application of FYM (Zaller & Koepke, 2004; Mandal et al., 2007; Okur et al., 2008; Bhattacharyya et al., 2012), vermicompost (Yadav et al., 2013), poultry manure (Yadav et al., 2013), biodynamic (Reeve et al., 2010) and panchagavya (Xu, 2001). The results from diverse soils and different crops are in conformity with the present findings (Liu et al. 2017).

**Conclusion**

The results revealed that, the soil microbial population (bacteria, fungi and actinomycetes), soil enzyme activities viz., dehydrogenase, alkaline phosphatase and fluorescein diacetate; and soil microbial biomass carbon were found higher under organic agriculture followed by conventional and/or biodynamic agriculture management. Similarly, soil microbial biomass carbon was found 30-45% and 33-42% higher under organic agriculture management as compared to conventional and biodynamic agriculture management, respectively. No significant effect of biodynamic agriculture management on soil microbial properties was observed. Among the different agriculture management practice examined, the organic agriculture management reflected as a viable technique in improving soil microbial properties. It is suggested that, further research is required to assess the potential of the organic agriculture towards nutrient mobilization through changes in soil microbial properties in different soils and diverse crops.

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**Conflict of interest**

All the authors declare that there is no conflict of interest.

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