International Journal of Research in Biosciences Vol. 4 Issue 2, pp. (8-20), April 2015 Available online at http://www.ijrbs.in ISSN 2319-2844

Research Paper

An investigation on effect of different agricultural practices on soil microflora: a potential indicator of soil fertility

***Chaudhry Smita¹ , Panwar Jyoti ¹ , Yasmin Tabassum ¹ , Sharma Sonika ¹ , Panwar Harsh ²**

¹Institute of Environmental Studies, Kurukshetra University, Kurukshetra-136119, Haryana, INDIA ²Guru Angad Dev Veterinary and Animal Science University (GADVASU), Ludhiana-141004, Punjab, INDIA

(Received December 12, 2014, Accepted March 17, 2015)

Abstract

Agricultural management practices strongly influence the structure and function of soil microbial community. The present study was aimed to assess the effect of (a) different fertilizer treatments i.e organic (vermicompost, VM) and chemical(CH) and (b) tillage regimes i.e conventional tillage(CT) and no-till(NT) on soil microbial population and biomass. Soil samples were taken from rhizosphere (Rh) and non-rhizosphere (NRh) of wheat crop planted in field plots and were evaluated for microbial biomass carbon (MBC) and microbial biomass nitrogen(MBN). A positive effect of organic fertilizers and no-tillage system was observed on the microbial biomass carbon and nitrogen content of soil. MBC and MBN was also significantly higher in rhizosphere as compared to non-rhizosphere in all the selected farming systems. The average microbial biomass C was found to be 964.0 μg g-1 and 662.4 μg g-1 for Rh and NRh region of vermicompost treated soil. MBC for chemical fertilizer amended soil was observed as 713.5 μg g-1 (Rh) and 542.8 5 μg g-1 (NRh) respectively. Significant differences were observed between soil microbial flora of different systems studied. Results of the study revealed that organic farming and conservation tillage practices restore more microbial flora and improve soil microbial properties, which are potential indicators of soil structure and fertility and hence effect the crop productivity.

Keywords: Chemical fertilizers, Microbial Biomass C, Microbial Biomass N, Tillage systems, Vermicompost.

Introduction

The expansion of modern agriculture with implementation of intense farming practices is amongst the greatest threat to soil biodiversity. Over the last quarter of the 20th century, range and abundance of many species associated with farmland have been reported to decline, leading to growing focus over suitability of current intensive farming practices. Soil microflora plays a crucial role in processes of both natural and managed ecosystems involving management of various biogeochemical cycles [1], contribution to plant health and nutrition $[2]$, soil structure $[3]$, soil fertility $[4]$ and suppression of plant pathogens ^[5]. Soil environment is strongly affected by the type of agriculture practice and hence soil microbial communities are likely to be affected. It is evident from earlier reports that intensive farming practices including tillage, monocropping and application of chemical fertilizers have detrimental effects on activity and occurrence of wide range of soil microflora $[6, 7]$.

Level of organic matter retained overtime by agricultural soil depends upon the type of farming

practice ^[8]. Minimum tillage systems such as no-tillage (NT) and diverse crop rotations are practices that could maintain and improve soil quality. Continuous cultivation along with frequent tillage results in rapid loss of organic matter. In contrary to this, minimum or zero tillage could maintain and improve soil quality ^[9]. Application of organic fertilizers is known to increase the nutrient status, fertility and productive potential of soil ^[10], promote soil structure formation ^[11] enhance soil biodiversity ^[12] and soil microbial activity $^{[13]}$ alleviate environmental stresses $^{[14]}$ and improve food quality and safety $^{[15]}$. Besides being affected by type of agriculture practice, soil microflora is distinct between rhizospheric and non-rhizospheric zone. Bacterial activities in non rhizospheric zone are reported to be depressed due to lack of a suitable energy source, supplied by plant roots in the vegetated soil. However, on other hand, microbial activities and microbe driven actions are much higher in rhizosphere zone than in non-rhizospheric zone ^[16]. Study of soil microbial biomass can be used to determine the effect of agriculture practice over soil health, much faster than the conventional methods based on estimation of total organic carbon or nitrogen [17].

India, Haryana has been well known in history since the ancient times, as being the cradle of Indian culture and agriculture. Agriculture sector continues to play a major role in the state economy contributing about 14.5 percent to its Gross Domestic Product (GDP) as well as providing employment to 51 percent of the work force (Haryana State Agriculture Policy, 2010). The state has emerged as a major contributor to the national pool of food grains. The manifold increase in agricultural production of Haryana has been revolutionary. Notwithstanding these impressive achievements, the state is facing problems of decreasing size of farm holdings, increasing soil salinity, harsh climate, imbalanced use of fertilizers and micro-nutrient deficiency. All these factors are adversely affecting productivity enhancement.

In order to maintain the soil sustainability, it is important to assess the effects of fertilizer applications on soil microbial spectra, which control nutrients cycling in the soil. An understanding of the effect of different agriculture practices over soil microbial community is important for the proper management and better output of farming systems. Although the role of microbes in agriculture is well documented, but there is still scanty information available on the effect of different agronomic practices over the soil beneficial microflora. More knowledge of short and long-term effects of various agricultural management practices is required to assess the contribution of these practices to sustainable land management. Hence, the present investigation was carried out with an aim to assess the impact of organic v/s inorganic farming practices and tillage v/s no-till systems on the dynamics of soil microbial population and microbial influenced parameters in the agricultural fields of North-West Haryana. Also a distinction was made between rhizosphere and non-rhizosphere microflora of mentioned systems.

Materials and Methods

Site selection

In order to make a comparative analysis, agricultural fields with different farming practices were selected from region of North West Haryana. Organic v/s inorganic farming system was available at agricultural fields of Gurukul, Kurukshetra (29°57'35"N & 76°48'25"E) whereas conventional tillage v/s no-tillage system was selected from experimental fields of Central Soil Salinity Research Institute, Karnal (29°42'29"N and 76°57'11" E). The state is divided into two agro climatic zones: North western part, suitable for rice, wheat, vegetable and temperate fruits and the south western part, supports high quality agricultural produce, tropical fruits, exotic vegetables and herbal and medicinal plants. Climate of the study area is tropical monsoonal and semiarid in nature with old alluvium soil, which is sandy loam (sand 61 %, silt 24% and clay 15%) and slightly calcareous in nature. The selected wheat fields have been continuously under the studied farming practices over a period of seven years. The organically treated fields were amended with vermicompost (100 kg/acre) (animal dung vermin-composted under field conditions for 90 days) as fertilizer source, while the inorganically treated fields were amended with mixture of urea (50 kg/acre), diammonium phosphate (DAP) (25 kg/acre) and zinc (5kg/acre). Under no tillage practice, crops were planted into undisturbed soil using zero till drill machine, while the conventional tillage consisted of two disk plowings and two diskings with a light harrow to level the soil and prepare the seedbed. Under both tilled and no tilled systems, fields were amended with mixture of urea (100 kg/acre) and DAP (50 kg/acre).

Soil Sampling

Soil samples were collected randomly in triplicates from rhizosphere and non-rhizosphere of both the

selected systems. The wheat crop was in shooting phase at the time of sampling. For rhizosphere soil, intact root system was dug out and clumps of soil loosely adhering to the roots were removed and collected whereas undisturbed soil core samples from non-rhizophere (0-15 cm) were collected using soil core sampler having stainless steel rings (5 cm diameter each). Three soil samples for each treatment were collected, thoroughly mixed, homogenized, air dried, sieved (<2mm) and immediately stored at 4°C in sealed plastic bags to prevent any loss of microbial parameters. The data generated from two systems was used to assess the impact of different agronomic practices on the dynamics of soil microbial populations and their activities in wheat fields.

Isolation of Bacteria and Fungi from Soil

Soil samples were immediately processed for isolation of both bacteria and fungi in their respective growth media. The serial dilution plate technique $^{[18]}$ was employed to enumerate the fungi and bacteria from rhizospheric and non-rhizospheric soil. Nutrient agar and Czapekdox agar medium was used for isolation of bacteria and fungi respectively. Plates in triplicate (dilutions, 10^{6-9} and 10^{2-4} for bacteria and fungi respectively) were incubated for 24 to 48 hrs at $37\pm1^{\circ}$ C for bacteria and 48 to 72 hrs at $25\pm1^{\circ}$ C for fungi. After incubation period, the colony forming units were counted and expressed as CFU/gram of soil.

Morphological and biochemical identification of bacteria and fungal isolates

Individual and morphologically distinct colonies were randomly picked and further streaked over nutrient agar plates to obtain pure isolates. Single, pure colonies were picked up and subjected to gram's staining, endospore staining and catalase test on the basis of Bergey's manual [19]. Distinct fungal isolates were plated over czapekdox agar and allowed to grow until formation of lawn of hyphae. Microscopic analysis of fungal strains was performed using lactophenol cotton blue staining after incubation for 4-5 days at 25° C \pm 1^oC.

Estimation of Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass carbon and nitrogen was estimated by fumigation-extraction method. 12.5 gram of moist soil sample in triplicate was extracted immediately after sampling by shaking for 30 min. with 50 ml of 0.5 M K_2SO_4 . Simultaneously, further triplicate samples of moist soil were fumigated for 24 h with ethanol-free chloroform (CHCl₃) at 25°C and then extracted with K₂SO₄ similarly. Microbial biomass C was calculated by measuring the difference in extractable organic C between the fumigated and unfumigated soils, using the equation : Biomass C = 2.64 \times E_C^[20], where E_C refers to the difference in extractable organic C between the fumigated and unfumigated treatments and 2.64 is the proportionality factor for biomass C released by fumigation extraction. Microbial biomass N was calculated using the equation: Biomass N= 1.85 E_N ^[21], where E_N = (total N from fumigated soil) - (total N from unfumigated soil). Results were expressed as mean ± SEM. Data was compared using Student's *t* test. Groups of data were considered to be significantly different if p < 0.05.

Results and Discussion

Effect of different farming practices on soil microflora

Effect on soil bacterial population

Total microbial count was recorded to be variable in comparative farming practices (Table 1). Vermicompost treated rhizosphere could support growth of $43x10^5$ CFU/gram, in comparison to $31x10⁵$ CFU/gram of chemical fertilizer treated soil. However, non-rhizosphere of both the systems showed comparatively lower colony count. Results from present study represented a clear increase in soil microbial population, with adoption of organic farming that may be attributed to the impact of organic amendment on the activity of microbes. Addition of higher inputs of organic matter as an energetic substrate, assures turnover of applied nutrients with activation of microbial communities. Our results are in line to earlier reports that supported instance of increased microbial density ^[22] upon incorporation of organic amendments. Furthermore, this increase can be supported with the fact that vermicompost used as organic amendment is a product of earthworm activity, which is known to promote microbial activity by fragmentation and conditioning of the substrate with an increase in surface area for microbial activity. Increase in microbial population has been reported in earthworm excreted or processed material than the parent material [23].

The bacterial population was higher under NT than the CT farming practice. The rhizosphere under NT system represented 53x10⁵ CFU/gram soil, which was significantly higher than 46x10⁵ CFU/gram of soil under tillage practice (Table 1). Similar trend was recorded in non-rhizosphere, although the difference was not as prominent as seen in rhizosphere. Earlier studies have shown no-till practice to stimulate both bacterial and fungal populations ^[24]. This promotion of microbial growth under NT system can be explained with the accumulation of crop residues on the soil surface that enriches soil organic matter in the surface layer with simultaneous increase in number of microorganisms.

Table 1: Effect of different fertilizer treatments and tillage regimes on bacterial population of the soil

Morphological and biochemical analysis of bacterial isolates of soil under different agronomic practices

A total of forty pure bacterial isolates, twenty from the each of the two selected farming practices, were subjected to morphological and biochemical analysis i.e. Gram's staining, endospore staining and catalase test, the results of which are shown in Table 2-5.

Table 2: Phenotypic characterization of 10 bacterial isolates of organic system on the basis of morphological and biochemical tests

Majority of the isolates obtained from soil with different fertilizer treatments were gram positive bacteria, while only a few were gram negative as shown in Table 2-3. Gram-positive bacteria have specialized activities in soil e.g. plant growth promotion and bioremediation, and they could be advantageous for agricultural use [25].

Furthermore, most of the isolated bacteria were rod shaped with different grouping patterns. All the bacterial isolates were found to be catalase positive. However, only 8 of the total 20 isolates were found to be endospore producing. The endospore is able to withstand a wide range of environmental conditions for very long periods and is an important component of many natural microbial communities [26]. Despite its apparent metabolic inactivity, endospore constantly monitors the nutritional status of its surroundings. However, little is known on the abundance of endospore in agricultural soils and how management provokes endospore formation ^[27]. Some comman bacteria may be found in both organic and inorganic fields but only those treated with organic amendment are able to make the proper use of soil microclimate or micronutrients [28].

Table 3: Phenotypic characterization of 10 bacterial isolates of inorganic system on the basis of morphological and biochemical tests

Table 4: Phenotypic characterization of 10 bacterial isolates of tillage system on the basis of morphological and biochemical tests

Table 5: Phenotypic characterization of 10 bacterial isolates of no-till system on the basis of morphological and biochemical tests

Out of twenty bacteria isolated from the selected tillage practices, 11 were found to be endospore producing (Table 4-5). Majority of the bacterial isolates obtained were gram positive with rod shaped morphological appearance. All the bacterial isolates were found to be catalase positive. The structure of the microbial community and enzyme activity gets affected by several factors, such as farming systems ^[29], plant species ^[30], tillage and crop rotation ^[31].

Effect on soil fungal diversity

Besides having a considerable difference in soil bacterial population, difference was also observed

between fungal genera of soil under different farming patterns. Upon lactophenol staining, the distinct fungi identified under vermicompost amended soil were *Aspergillus sp.*, *Penicillium sp*., *Alternaria sp*. and *Fusarium sp*. with a predominance of *Alternaria* and *Penicillium* (Figure 1(a-c)). Although presence of all the four fungal genera was also seen under chemical fertilizer treated soil, a notable difference existed for *Penicillium sp*. with presence of only one cultured colony. From the aforesaid results, it is quite clear that the vermicompost fortified soil provides a favourable environment for *Penicillium sp*. A general trend of elevated fungal activity under organic farming has also been documented by previous studies [32, 33]. Presence of *Penicillium* and *Aspergillus* has been reported to be beneficial for plant growth promotion [34]. An isolate of *Penicillium* identified as *P. citrinum* has been reported to promote plant growth by gibberellins production [35] *. Aspergillus fumigates* has been reported to be common inhabitant of soil, responsible for providing nutrition via biodegradation. A rich population of fungi was also obtained in both conventional tillage and no-tillage system. The distinct fungal strains identified were *Alternaria, Aspergillus, Penicillium and Fusarium*. *Alternaria* was predominant in both the tillage systems. However, more variants were observed at species level under NT practice, as can be inferred by candidate isolates with different sporulation pattern (Figure $1(d-f)$).

Figure 1: Fungal isolates observed in the soil with different farming practices (a) *Aspergillus sp.* **(b)** *Penicillium sp.* **(c)** *Fusarium sp***. (d-f)** *Alternaria sp.* **with different sporulation pattern**

Effect of different fertilizer treatments on soil microbial parameters

Microbial Biomass Carbon

Organically treated fields exhibited a significant variation in soil MBC as compared to inorganic fertilizer amended fields (Figure 2(a-c). MBC in non-rhizophere of chemical fertilizer supplemented soil (542.8 µg g⁻¹) was significantly (p<0.05) less than the recorded value of rhizosphere soil i.e.713.5 μg g⁻¹. MBC of vermicompost amended soil also had higher value of MBC in rhizosphere than that of non-rhizosphere soil (964.0 μg g⁻¹ and 662.4 μg g⁻¹ respectively). On comparative analysis of the data between inorganically and organically treated fields, MBC of rhizosphere of vermicompost supplemented fields was significantly (p<0.05) higher than the non-rhizosphere soil of inorganic fields, with an increase of around 35 percent from inorganic to organic plots. Although an increase of about 22 percent was recorded between same systems in non-rhizosphere, but the difference was not statistically significant (Figure 2(c)).

Shift from chemical to vermicompost amendment provided a recovery in soil microbial biomass carbon, as documented in the results of present study. Compost addition significantly increased microbial activity, expressed through the contents of the C and N biomass ^[36]. A positive effect of organic fertilizers on microbial biomass nitrogen and the carbon content in the soil was also observed and reported by Cerny et al. (2008)^[37].

Figure 2: Microbial biomass carbon in chemical fertilizer and vermicompost supplemented soil, along with Comparative analysis of microbial biomass carbon between chemical fertilizer and vermicompost amended soil. (* p=0.0149, * p<0.0001)**

Stimulation of microbial biomass and activities by organic carbon inputs has been well documented in various organic substrates ^[38, 39]. Our results with respect to soil microbial biomass carbon are in line with the study of Santos *et al.* (2012)^[40], which reported higher soil microbial biomass carbon at all depths under organic farming system in comparison to plots maintained under conventional farming, that can be attributed to organic inputs from compost, which promotes greater amounts of carbon.

Microbial Biomass Nitrogen

Microbial biomass nitrogen values of soil treated with different fertilizers has been shown in Figure 3(a)-(b). Data represented in Figure 3(a), clearly indicates that rhizosphere soil had significantly $(p< 0.05)$ more microbial biomass nitrogen (49.97 µg g⁻¹) as compared to the MBN value of nonrhizosphere (39.49 µg g⁻¹). Organically treated fields also exhibited higher values of MBN in rhizosphere in comparison to the soil of non-rhizosphere, as can be inferred by values of 68.85 µg g⁻¹ and 46.39 μg g⁻¹ respectively. Further, it is evident that MBN of rhizosphere of organically amended fields was significantly (p<0.05) higher than that of chemical fertilizer treated fields. However, there was no significant difference in MBN values of non-rhizosphere of these treatments (Figure 3(c))

High amounts of organic inputs result in high microbial biomass ^[41]. Our results with regard to persistence of significantly more soil microbial biomass carbon and nitrogen in organic farming practice under wheat crop can be further corroborated with earlier reports, who too reported presence of more organic carbon and nitrogen in organically managed soils under different plantations viz. tomato $^{[42]}$, vineyard $^{[43]}$, pea-durum wheat- tomato rotation $^{[44]}$.

On comparative evaluation of the data obtained, it can be postulated that organic farming could be a better alternative for maintaining soil health and fertility. The differences in microbial biomass carbon and nitrogen between treatments may be due to differences in rates of leaching of particular nutrients or microbial immobilization of these nutrients. Decreased leaching of nitrates has been reported from compost-treated soils [45]. Since there were more soil microorganisms in soils treated with vermicomposts, they could sequester nutrients and use them for metabolic activities.

Figure 3: Microbial biomass nitrogen in chemical fertilizer and vermicompost supplemented soil (* p=0.039, ** p=0.0011) along with comparative analysis of microbial biomass nitrogen between chemical fertilizer and vermicompost amended soil (* p=0.0002)**

Effect of different tillage practices on soil microbial parameters

Microbial Biomass Carbon

Soil MBC was determined in samples collected from rhizosphere and non-rhizosphere of fields under conventional tillage and no-tillage farming practices and the results obtained are shown in the form of bar chart (Figure 4(a)-(b)). Microbial biomass carbon had a higher range of values in NT system as compared to the conventional tillage practice in both the zones (Figure 4(c)). Microbial biomass carbon in tilled rhizosphere soil was 471.2 µg g⁻¹, however, MBC in non-rhizosphere under same treatment was significantly (p<0.05) less than that of rhizosphere soil, as can be recorded by value of 108.3 μg g⁻¹. The value of MBC for rhizosphere soil of NT farming practice was much higher than that of non-rhizosphere soil (642.8 µg g⁻¹ and 315.1 µg g⁻¹ respectively).

Our results are in accordance with Wright et al. (2005)^[46], who reported the higher values of microbial biomass carbon and microbial biomass nitrogen under no-till surface soils. Other studies have also suggested that there may be a significant long-term increase in soil microbial biomass throughout the topsoil in various reduced tillage systems $[47, 48]$. The general increase of microbial biomass under NT over CT, especially under tropical/subtropical conditions, could be attributed to several factors, such as a lower temperature, higher moisture content, greater soil aggregation and higher C content. The lack of a major disturbance event with NT likely provides a steady source of organic C to support the microbial community compared to CT where a temporary flush of microbial activity with each tillage event results in large losses of C as $CO₂$.

Figure 4: Microbial biomass carbon in tilled soil (* p=0.0179), No-till soil (p=0.0042) along with comparative analysis of microbial biomass carbon between soil under tillage and No-till agricultural practice (** p=0.0017).**

Microbial Biomass Nitrogen

MBN in rhizosphere of tilled soil (29.93 μ g g⁻¹) was significantly (p<0.05) higher than the MBN value of non-rhizosphere, which was recorded as 17.41 μ g g⁻¹ (Figure 5(a)-(b)). Microbial biomass nitrogen of

non-rhizosphere soil under no-till practice was much lesser than that of rhizosphere (21.90 µg g⁻¹ and 45.19 μg g⁻¹ respectively). On comparative analysis of the data between conventional tillage and notill soil, microbial biomass nitrogen of both rhizosphere and non-rhizosphere of NT system was higher than that of CT system (Figure 5(c)).

Results of the study with respect to estimation of microbial biomass carbon and nitrogen in the two tillage systems clearly indicates presence of more MBC and MBN in rhizosphere and no-till practice as compared to the non-rhizosphere and tillage practice respectively. These results are in confirmation with earlier findings of Mohammadi (2011)^[49], who reported enhanced microbial biomass carbon in no-till system as compared to the other tillage practices. Besides having effect on microbial parameters, no-tillage also demonstrated highest rate of grain yield.

On similar lines, Mathew *et al.* (2012)^[50] reported that no-till practices improved both physio-chemical and microbiological properties of soil. Over the long term, the variation in biomass N between tillage systems corresponds with crop residue distribution. This may be attributed to immobilization of fertilizer N which in turn, is associated with the incorporation of recent crop residues or levels of labile organic matter in the surface soil.

Conclusion

Soil composition and health reciprocates the diversity and number of soil microbial spectra and further affects efficiency and retention of nutrients within an agro-ecosystem. Evaluation of key microbial indicators such as microbial biomass may provide insight into the long-term fertility status of the soil ecosystem processes. Results of the present study clearly indicated that the application of chemical fertilizer treatments and tillage practices profoundly affected the soil microbial population and related parameters. Soils under organic and no-till agricultural practice exhibited the higher values of microbial biomass nitrogen and carbon which proved that a greater microbial biomass could be achieved by these practices, which is attributed to higher nutrient availability for microorganisms in these treatments. Furthermore, there would be greater cycling and fluxes of nutrients through the microbial biomass in these soils. The formation and stabilization of macro-aggregates in NT soil is likely to be a key mechanism for the protection and maintenance of microbial habitat. The application of organic fertilizer resulted in most pronounced growth of microbial population as compared to inorganic treatment. The vermicompost amendment in organic farms provided a significantly greater input of organic carbon, which increased the microbial population. Application of organic manure and zero tillage has been found to be more eco-friendly, economically viable and ecologically sound option for promotion of microbial growth and soil health. This study is significant in providing inputs for natural resource management which has to be an important strategy for accelerating and sustaining agricultural growth in Haryana, a state in India, which is predominantly an agriculture economy with diverse cropping patterns.

Acknowledgement

Jyoti Panwar thanks Kurukshetra University, Kurukshetra for her Ph.D scholarship support as URS.

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