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Functional diversity of organic and inorganic manured soils under cotton-wheat cropping sequences

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Abstract
Community level physiological profile (CLPP) assays, which assess a microbial community, has the capacity to metabolize specific sole carbon sources for study of environmental soil samples. Analysis of soils after ten years of application of different organic manures and inorganic fertilizers under cotton-wheat cropping system at CCS H.A.U Hisar shows that there was a buildup of higher microbial functional diversity than those in conventional farming systems. In this study, we made an attempt to find out the effect of organic manuring and integrated nutrient management on microbial communities and BIOLOG assay. Functional microbial diversity on the basis of sugar and amino-acid utilization pattern was also studied in these soils and it was found that in inorganically fertilized, integrated managed and organically manured soils formed two different groups indicating the different microbial activity in these treatments.

Keywords: CLPP, organic manures, vermicompost, cotton-wheat cropping system and inorganic fertilizers

Introduction
The organic production system aims at supporting and sustaining healthy ecosystems, soil, farmers, food production, the community, and the economy. Reduction and elimination of the adverse effects of synthetic fertilizers and pesticides on human health and the environment is a strong indicator that organic agriculture is gaining worldwide attention. Organic fertilizers are environmentally friendly, since they are from organic sources. The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable food production. To build up or to maintain the level of soil organic matter in agricultural soil, use of organic amendments or integrated use of chemical fertilizers along with organic amendments is being advocated. The complementary use of chemical fertilizers, biofertilizers, organic manures and crop residues can ensure long-term nutrient supply and soil productivity (Stockdale *et al.*, 2001) [11].

Food and environmental safety are the main reasons for a rise in popularity of organically produced food and the demand for organic food has been increasing world over (Thompson, 1998) [12]. But, the maintenance of adequate soil fertility is a major challenge in organic and low-input farming systems (Mader *et al.*, 2002) [6]. Nitrogen is supplied in organic form through manures, crop residues and green manures rather than as inorganic fertilizers as in conventional farming systems. Use of organic amendments as a source of plant nutrients has often been associated with desirable soil properties including improved soil structure, water holding capacity, cation exchange capacity, build up of soil organic matter and proliferation of beneficial soil organisms (Bulluck *et al.*, 2002; Garcia-Ruiz *et al.*, 2008) [1, 3]. Soil chemical characteristics are affected by amendment of soil with organic manures or chemical fertilizers or their combination. Organic amendments provide advantage over chemical fertilizers because nutrients other than N such as P, K, Ca, Mg, S and other micronutrients are always present in organic manures (Sharma *et al*., 2011) [9]. The use of recycled organic wastes such as farmyard manure and other organic amendments such as poultry waste, press mud and non-edible oil cakes can result in enhanced soil organic matter level which improves soil physical, chemical and biological attributes compared with synthetic chemical fertilizers (Singh, 2010) [2]. Use of organic manures, adoption of recommended soil and crop management practices including use of crop residues, harnessing of biological nitrogen fixation through the cultivation of legumes can help in soil organic C sequestration (Lal, 2002) [5]. The present study was undertaken to compare soil microbial activities in organic, integrated and conventional farming systems.

Soil samples from different fields fertilized with, organic manures a combination of organic manures and inorganic fertilizers, inorganic fertilizers and unfertilized soils were analyzed and compared.

Materials and Methods

Experimaental design and Treatments

Soil samples from fields fertilized only with organic manures, combination of organic and inorganic nutrient sources and only inorganic fertilizers were collected from 0-15 cm soil layer in crop rotation systems from CCS H.A.U Hisar.
The cropping pattern and management was as follows:

T_1	Absolute control (No fertilizer)
T_2	Check (RDF through chemical fertilizer)
T_3	RDN through FYM
T_4	RDN through vermi-compost
T_5	RDN through Neem cake
T_6	RDN through 1/3 FYM +1/3 vermi- compost+ 1/3 Neem
T_7	Phospho-compost + N through organic source
T_8	Phosphovermi-compost + N through organic source
T_9	75% RDN through vermicompost + *Azotobacter* + P requirement through rock phosphate
T_{10}	T6 + cowpea intercropping in cotton and mustard intercropping in wheat

Soil samples amended with organic and inorganic manures from last ten years were drawn from research farm CCS H.A.U Hisar. The soil samples were sieved through 2 mm sieve and stored at 4°C. The soil was moistened to 60 per cent water holding capacity (WHC) and incubated at 30° C for 10 days to permit uniform rewetting and to allow microbial activity to equalibriate after the initial disturbances. Sub-samples of each soil were air-dried and ground for chemical analysis. Soil samples were analysed functional diversity.

Substrate utilization in Biolog for analysis of CLPP

Basal Medium

Component	Amount (g/L)
K_2HPO_4	1.0
Cacl $_2$.2H$_2$0	0.1
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.05
$Na_2MoO_4.2H_2O$	0.005
Bromocresol purple	0.05
Ph	7.0

The Biolog microplate comprises 22 different sugar and 9 amino-acids as a substrate and a control well without a carbon source (Table 1). Serial dilution of each soil sample was made and 100 µl of diluted soil sample was added in a well of microtitre having sugar and medium. The plates were incubated at 20°C in the dark. Development of color was measured after every 24 h for 5 days using an Elisa plate reader at 592 nm and substrate utilization was calculated.

Table 1: Sole carbon sources in Biolog microplate

C- sources	
Carbohydrates	**Amino acids**
Dextrose	L-Alanine
Fructose	Glycine
Ribose	L-Serine
Galactose	L-Treonine
Xylose	L-Tryptophan
Mannitol	L- Arginine hydrochloride
Soribitol	L-Asparagine
Dulcitol	L-Histidine hydrochloride
Rhamnose	4-Hydroxy praline
Inositol	
Sucrose	
Lactose	
Maltose	
Mannose	
Adonitol	
L- Arabinose	
Cellobiose	
Inulin	
Melibiose	
Raffinose	
Salicin	
Trehalose	

Results and Discussion

Substrate utilization in Biolog for analysis of CLPP

To find out the functional microbial diversity of organically and inorganically manured soils each soil samples were serially diluted and 100 µl of diluted soil samples were added in a well of Biolog plates having 22 sugar, 9 amino-acids and medium. The plates were incubated at 20°C in the dark. Development of color was measured after every 24 h for 5 days using an Elisa plate reader at 592 nm. and substrate utilization was calculated. There is no development of color in Biolog plates after 24 hours (Plate-1) but plates showed maximum development of color after 72 hours (Plate-2).

Functional microbial diversity of organically and inorganically manured soils at CCS H.A.U, Hisar.

Functional microbial diversity of organically and inorganically manured soils in cotton-wheat rotation system at CCS H.A.U, Hisar is presented in Figure 1. There are two major group at 36% similarity coefficient. Major group 1 and 2 includes different treatments with FYM, VC, NC and chemical fertilizers and form separate subgroup. Subgroup 1-I with the addition of phosphocompost and vermicompost along with organic source has no effect on microbial population and showed 100% similarity with treatments TR 7 and TR 8. Treatment 5 having RDN through neem cake showed 90% similarity with the above treatments. Treatment containing RDF through chemical fertilizers, TR 2 is out grouped in major group 1 from rest of the treatments TR 5, TR 7 and TR 8 at 66% similarity coefficient. However, in major group 2TR 4 and TR 9 showed 95% similarity with treatment RDN through VC+ *Azotobacter*+ P requirement through rock phosphate indicating that there is no change in micro flora in these treatments. The cowpea intercropping in cotton and mustard intercropping in wheat along with RDN through 1/3 FYM, 1/3 VC and 1/3 NC as shown in treatments 6 and 10 showed 88% similarity coefficient. The control TR 1 is out grouped in major cluster II from rest of the treatments at 55% similarity coefficient.

Garland and Mills (1991) [4] studied the Biolog redox technology based on tetrazolium dye reduction as an indicator of sole carbon source utilization and this technique was evaluated as a rapid method to characterize and classify heterotrophic microbial communities. Direct incubation of whole environmental samples (soil and rhizosphere) in Biolog plates containing 95 separate carbon sources produced community dependent patterns of sole carbon source utilization. Principal component analysis of color responses quantified from digitized images of plates revealed distinctive patterns among microbial habitats and spatial gradients within soil. Correlation of the original carbon source variables to the principal components gave a functional basis to distinctions among communities. Narula *et al*. (2002) [7] studied the microbial population in soil influenced by the amount and type of various compounds entering soil through plant litter, root exudates and management factors like mineral and organic fertilizers under various climatic conditions. Number of various groups of microflora and substrates used by Biolog were studied to assess the metabolic capabilities of various groups of microbes from soils of long term fertilization experiment, having different mineral and organic fertilizer treatments in unplanted and planted soils with two crops viz., *Secale cereale* and *Medicago sativa*. In substrate utilization test (Biolog), maximum utilization was found for sugars followed by amino acids in *Secale* whereas in *Medicago* sugars utilization pattern were followed by carboxylic acids, which was confirmed by plate count method also as the rhizosphere of *Medicago sativa* and *Secale cereale* had high number of total microorganisms, nitrogen fixers and phosphate solubilizers as compared to bulk soils. Microbial counts were specially higher in treatments of NPK+FYM and NPK in *Secale.*

Chakraborty *et al*. (2010) [2] reported soil quality and community-level physiological profiles (CLPP) of bacteria in soil under a long-term (37 years) trial with either exclusive inorganic fertilizers or fertilizers combined with farmyard manure cultivated with jute– rice– wheat system. The treatments consisted of 100% recommended dose RD of NPK, 150% RD of NPK, 100% RD of N, 100% RD of NPK+FYM (10 t ha^{-1} $year^{-1}$), and untreated control. Long-term application of 150% (RD) of NPK lowered the soil pH considerably while the soils in the other treatments remained near neutral. The 100% RD of NPK + FYM treated plot showed significantly highest accumulation of organic carbon, total nitrogen, microbial biomass carbon, basal soil respiration and fluorescein diacetate hydrolyzing activity among the treatments. CLPP analysis in Biolog ecoplates revealed that utilization of carbohydrates was enhanced in all input treated regimes, while the same for polymers, carboxylic acids, amino acids and amines/ amides were similar or less than the untreated control. However, within these groups of carbon sources, heterogeneity of individual substrate utilization between treatments was also noted. The addition of organic supplements showed significantly increased microbial biomass carbon and microbial activity but input of nutrient supplements, both inorganic and organic, only marginally affected the overall substrate utilization pattern of soil microorganisms. Sagar *et al*. (2017) [8] studied the functional diversity of soil bacteria from organic agro ecosystem. The organic farming enriches soil fertility and biodiversity, as well as, less dependent on external high inputs. Thus organic farming offers promise of achieving ecological, economic and social stability in food production system.

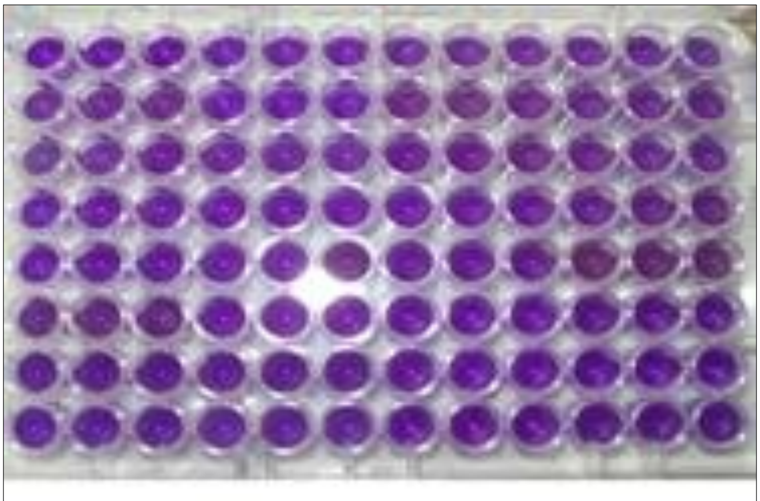

Plate- 1 Control

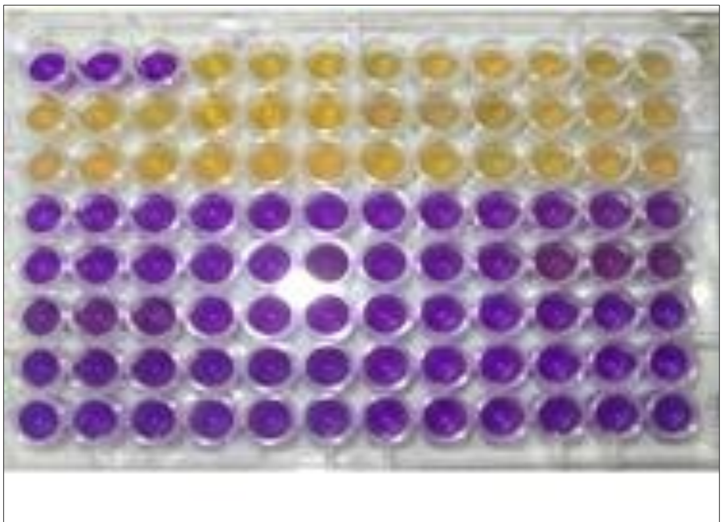

Plate- 2 Development of color in Biolog Plate after 72 hours

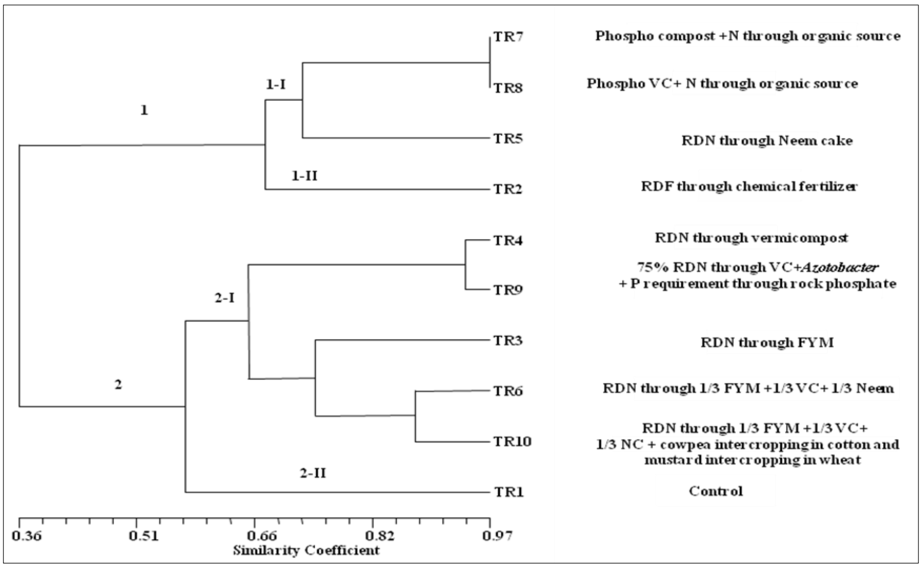

Fig 1: Functional microbial diversity of organically and inorganically manured soils in cotton-wheat rotation system at H.A.U

Conclusion

Combined applications of organic and inorganic sources of nutrients are more productive and sustain soil fertility. All organic manure increased microbial functional diversity in organic farming system.

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