

IMPACT OF LONG-TERM ZERO-TILLAGE AND CROPPING SYSTEM ON CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SOIL

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Abstract– Long-term tillage methods and crop rotations can change the physico-chemical and microbiological properties of soil. Under different climatic conditions, the tillage system may have an impact on soil qualities. In the present investigation, soil microbial diversity at different depths was studied in long term tillage experiment continued since 1997-98 (sorghum-wheat), 2014-15 (pearl millet-wheat), 2010-2011 (rice-wheat) and 2006-07 (guar-wheat) that was carried out in sandy loam, sandy, loamy sand and sandy textured soil at Hisar, Rewari, Karnal and Mahendragarh (Haryana) during 2017-18, respectively. Adoption of ZT practice positively affected EC, pH, Total NPK content, viable counts, enzymatic activities and functional diversity of microbes. As a result, ZT appears to be a successful method for improving the physico-chemical and microbiological properties of soil, which could lead to increased soil health and crop productivity.

INTRODUCTION

Soil quality highly depends on its structure, natural productivity and human influence and tillage is one of the major management practices affecting soil physical parameters. It is the mechanical disturbance of the soil through plowing, cultivation or digging and has been used by the farmers since ancient time. Tillage systems influence physical, chemical and biological properties of soil and have a major impact on productivity and sustainability. Conventional tillage practices increase the soil erosion and decrease the organic matter in soil which may adversely affect long-term soil productivity. Sustainable soil management can be practiced through conservation tillage (including ZT), high crop residue return, and crop rotation (Hobbs *et al.*, 2008). Studies conducted under a wide range of climatic conditions, soil types and crop rotations showed that soils under ZT have significantly higher soil organic matter contents compared with conventionally tilled soils (Alvarez, 2005). Conventional tillage can lead to soil microbial communities dominated by aerobic microorganisms, while conservation tillage practices increase microbial population and activity as well as microbial biomass (Balota *et al.*, 2003). For

sustainable agriculture, soil microflora is essential as its activity subsidizes to increasing agricultural production.

Microbial activity responds quickly to disturbances in a shorter period of time than other parameters. As a consequence, microbiological properties such as microbial biomass and different soil enzyme activities have been suggested as potential indicators of soil quality and fertility (Saviozzi *et al.*, 2001) because of changes in soil management quickly. The effect of tillage on soil microbial populations have generally been studied by comparing microbial numbers, soil microbial community (Klikocka *et al.*, 2012) and enzyme activities (dehydrogenase, phosphatase, cellulose and urease). Enzymes are responsible for carrying out mineralization of different soil nutrients such as phosphatase for P, urease for N and dehydrogenase represents total microbial activity of soil.

MATERIALS AND METHODS

Collection and preparation of soil samples: To study the effect of conservational practices on various properties of soil under different cropping systems, soil samples (0-15 and 15-30 cm depths) were collected from Agronomy Research Farm, CCS

Haryana Agricultural University, Hisar, Rewari, Karnal and Mahendragarh fields after harvesting of wheat in 2017, having sorghum-wheat, pearl millet-wheat, rice-wheat and guar-wheat cropping system under continuous zero and conventional tillage.

Characterization of Physico-chemical properties

Soil EC, pH, CaCO₃, Total N, P and K content: Five-gram soil was combined with 12.5 ml distilled water to test the pH and EC (electrical Conductivity) of different soil samples. It was shaken for 30 minutes and the pH of soil suspension was measured with Systronics 331 pH meter at room temperature, and EC was measured with Naina electrical conductivity meter. Calcium carbonate (CaCO₃) content in different soil samples was determined by the rapid titration method. Total nitrogen, phosphorous and potassium content in different soil samples was estimated by standard scientific methods.

Enumeration of microorganisms: Viable counts of bacteria and fungi in different soil samples were determined by serial dilution making and plating on respective media.

Soil Enzyme Activities: Soil dehydrogenase, alkaline phosphatase, cellulase and urease activities were determined by the standard scientific methods.

Functional diversity of different microorganisms using CLPP: Biolog microplate comprising of 22 different sugars and 9 amino-acids as a substrate and a control well without a carbon source was used to study functional diversity of different microorganisms. Serial dilution of each soil sample was made and 100 µl of diluted soil sample was added in a well of microtitre plate having sugar basal medium and the plates were incubated at 20±2°C in dark. Development of color from blue to yellow was measured after every 24 h for 5 days using an Elisa plate reader at 592 nm and substrate utilization was calculated.

RESULTS AND DISCUSSION

Physico-chemical properties

EC, pH, CaCO₃: The data on pH, EC and CaCO₃ of soils under conventional and zero- tillage systems under different crop rotations presented in Table 1 indicated that shifting from conventional to zero-tillage had increased EC, pH and CaCO₃ content. All

the soils were non-saline as the EC at different locations was lower than the critical limit of 0.8 dS m⁻¹. EC of soils under conventional-tillage ranged from 0.12 to 0.35 dS m⁻¹ at 0-15 cm depth while 0.14 to 0.39 dS m⁻¹ at 15-30 cm depth whereas EC of soils under zero- tillage ranged from 0.13 to 0.36 dS m⁻¹ at 0-15 cm depth while 0.15 to 0.41 dS m⁻¹ at 15-30 cm depth. The pH of soils varied from 7.3 to 8.7 at different locations under different systems with the values ranging between 7.7-8.1 at 0-15 cm depth and 7.3-7.7 at 15-30 cm depth under conventional-tillage whereas pH of different soils upon adoption of zero-tillage ranged between 7.7-8.7 at 0-15 cm depth and 7.5-7.9 at 15-30 cm depth. Although, a slight change in pH was observed upon adoption of zero-tillage practice but no definite trend was observed between the two systems at different locations. CaCO₃ content of different soil samples varied from 0.27-0.57 % at 0-15cm depth while 0.23-0.56 % at 15-30 cm depth under conventional tillage whereas with the adoption of zero-tillage system CaCO₃ content ranged from 0.36-0.62% at surface soil which decreased in a range of 0.31 to 0.61% at subsurface soil.

Tillage practices can influence the chemical characteristics of soil and conservational tillage affect the soil pH and soluble salt contents at different depths due to buffering capacity of soils based on carbonate contents which may resist change in pH of soils during organic matter decomposition under zero-tillage practices. These results are supported by the observations of Roldan *et al.* (2005) that EC was not affected by the tillage practices. Gholami *et al.* (2014) reported that ZT had highest EC value compared to CT because lower electrical conductivity of soil under the ZT system compared to CT pertained to the enhanced water movement in the soil that improved soil aggregate development. Kumar *et al.* (2018) observed decreased soil EC under CT that might be due to opening and aeration of the top soil layers which allowed increased leaching to occur at surface soil.

Similar observations have been reported by other researchers. Asenso *et al.* (2018) observed no significant difference on soil pH at 0-40 cm depth under zero-tillage that might be due to the fact that no liming material was applied as part of treatment.

The CaCO₃ content of different soil samples was affected by different crop rotations under conventional and zero-tillage to different extent, in present study and similar findings have been

Table 1. Effect of conventional and zero tillage on soil EC, pH, CaCO₃ and soil organic carbon under different crop rotations

Crop-rotations Locations	Tillage	EC (dS m ⁻¹)		pH		CaCO ₃ (%)	
		Depth (cm)					
		0-15	15-30	0-15	15-30	0-15	15-30
Sorghum-Wheat (Hisar)	CT	0.3	0.33	7.7	7.3	0.54	0.48
	ZT	0.32	0.35	7.8	7.5	0.57	0.52
Pearl-millet-Wheat (Rewari)	CT	0.12	0.14	7.8	7.7	0.27	0.23
	ZT	0.13	0.15	8.2	7.9	0.36	0.31
Rice- Wheat (Karnal)	CT	0.35	0.39	7.7	7.4	0.57	0.56
	ZT	0.36	0.41	7.7	7.6	0.62	0.61
Guar-Wheat (Mahendragarh)	CT	0.2	0.18	8.1	7.6	0.35	0.31
	ZT	0.22	0.2	8.7	7.6	0.37	0.33

reported in literature also. Neugschwandtner *et al.* (2014) reported increased calcium carbonate at 30–40 cm depth because the loss of CaCO₃ was reduced by conservation tillage due to greater retention of water in the soil profile. Celik *et al.* (2017) observed that calcium carbonate content of the soil was not significantly different within 0-30 cm depth, may be due to the tillage practices did not cause to accumulate calcium carbonate content within 30 cm of the soil surface.

Total N, P and K: Total nitrogen (N), phosphorus (P) and potassium (K) content of different cropping systems are important for sustaining crop productivity and food security and a large volume of literature is available which supports that long term ZT increases the total N, P and K content of soil at surface layer as compared to CT under different cropping systems (Neugschwandtner *et al.*, 2014).

In present study, conservational tillage at different locations was found to affect total N, P and K content under different crop-rotations varying in soil texture and higher total N, P and K content was observed under ZT system (Table 2). The total N, P,

and K content of RWCS was higher in upper layer than bottom layer with the values 0.061-0.052, 0.39-0.34 and 0.99-0.85 % in ZT at 0-15 and 15-30 cm depth, respectively, while respective values under conventional tillage were 0.055-0.047, 0.33-0.28 and 0.89-0.82. These results are consistent with the findings of Dorr de Quadros *et al.* (2012) reported significantly higher total N and P content in the no-tillage system because of high microbial diversity and high accumulation of soil organic matter.

Microbiological properties: No-tillage or conservation tillage preserves or enhances soil quality by maintaining soil structure and moisture, growing soil organic matter, and supplying soil microbes with a home. Most notably it is the bacteria that’s why soil microbes are the earth’s workhorses. To study the effect of tillage practices under different cropping patterns, the microbial count in soil samples was determined to know total viable count of bacteria and fungi. The microbial population increased by adoption of zero-tillage as compared to conventional tillage and was higher in surface soil under both systems.

Table 2. Crop-rotations from 0.26 to 0.26 ice y.l 3.1-6.6 tillage system in .24-0.43% at subsurface layer of soil. Effect of conventional and zero tillage on total N, P and K content of soil under different crop rotations

Crop-rotations Locations	Tillage	Total N (%)		Total P (%)		Total K (%)	
		Depth (cm)					
		0-15	15-30	0-15	15-30	0-15	15-30
Sorghum-Wheat (Hisar)	CT	0.05	0.045	0.23	0.16	0.66	0.58
	ZT	0.061	0.052	0.26	0.18	0.71	0.63
Pearl-millet-Wheat (Rewari)	CT	0.039	0.033	0.16	0.13	0.33	0.31
	ZT	0.041	0.037	0.24	0.18	0.35	0.32
Rice- Wheat (Karnal)	CT	0.055	0.047	0.33	0.28	0.89	0.82
	ZT	0.061	0.052	0.39	0.34	0.99	0.85
Guar-Wheat (Mahendragarh)	CT	0.044	0.04	0.25	0.18	0.41	0.35
	ZT	0.049	0.042	0.33	0.28	0.44	0.39

Microbial count: The microbial count in soil samples was determined to know the total viable count of bacteria and fungi for studying the impact of tillage practices under different cropping patterns. Compared with traditional tillage, the microbial population increased by implementing zero-tillage and was higher in surface soil under both systems. Bacteria and fungi are a very significant functional group of soil species and involved in many biogeochemical processes.

In present study the effect of tillage practices on microbial groups under different cropping patterns was studied and population of bacteria and fungi was observed relatively higher in zero tilled soil at 0-15 cm depth compared to conventional tilled soil and bacteria were most abundant under all systems. The effect of tillage practices on total bacterial population is presented in Figure 1 revealed that bacterial population remained higher at surface soil and total bacterial count in different soil samples ranged from 4.82 to 8.36×10^6 and 4.41 to 7.42×10^6 cfu g^{-1} soil at 0-15 and 15-30 cm depths, respectively, under conventional system with corresponding values 5.22 to 8.82×10^6 and 4.69 to 7.94×10^6 cfu g^{-1} soil under zero-tillage.

There was significant increase in fungal count and maximum in rice-wheat cropping system at surface layer (14.82×10^3 cfu/g) which further decreased up to subsurface layer (11.56×10^3 cfu/g) upon adoption of zero tillage practices while guar-wheat cropping pattern had lower count at surface and subsurface layer 12.48×10^3 cfu/g and 9.82×10^3 cfu/g respectively and similar trend was observed under two cropping pattern at surface and subsurface layer in conventional tillage.

Such findings are consistent with Dorr de

Quadros *et al.* (2012)'s observations that microbial diversity in the no-tillage environment was substantially higher, as soil management affected soil biodiversity and abundance of individual organisms. Under the conservational agriculture method. Dongre *et al.* (2017) attributed comparatively higher bacterial and fungal counts at 0-15 cm depth to submerged conditions in a deeper layer.

Enzyme activity: Soil microbial processes are regulated by different enzymes and are influenced by many factors of physical, chemical and environmental significance. Enzymes in soil are biologically important because they affect plant nutrient availability and all microbial activities are directly linked to the various enzymatic activities produced extracellularly by specific microorganisms or plant roots. In soil, the principal sources of enzymes are microorganisms, active roots, and dead cells. Soil enzymatic activity is a delicate marker of the impact on microbial functions of ecological factors. In this study, the effect of soil management practices on different enzymatic activities under different crop-rotations at different locations *viz.* Dehydrogenase, alkaline phosphatase, cellulase, and urease were studied, and different enzyme activity was observed higher in the 0-15 cm layer compared to the 15-30 cm layer.

Measurement of dehydrogenase activity (DHA) is one of the analyzes of the overall soil condition, being a highly sensitive indicator of environmental changes (Galazka *et al.*, 2017). It is closely related to the cycles of carbon and nitrogen, and the biological oxidation of organic soil (Blońska *et al.*, 2016). The activity of dehydrogenase represents the complete oxidizing activity of soil microflora and in present

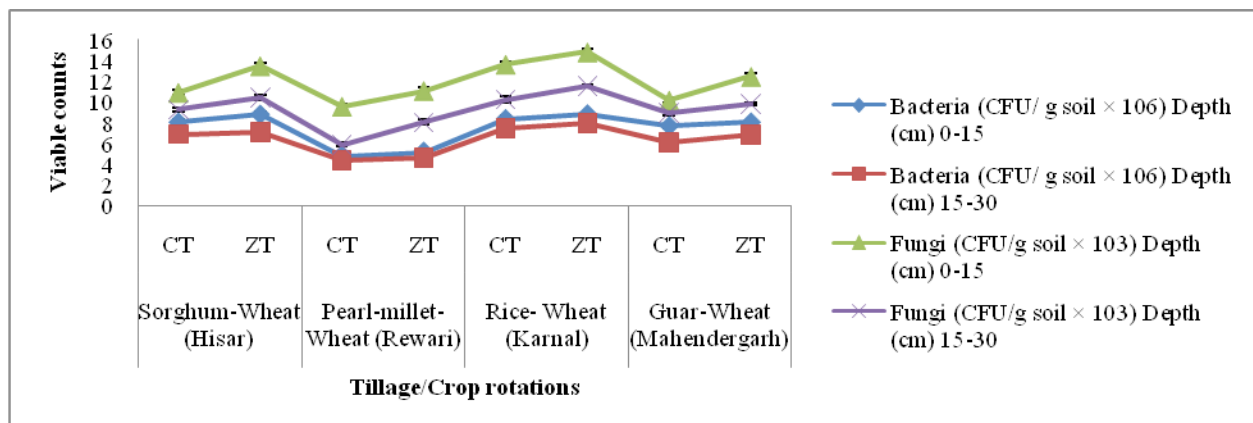


Fig. 1. Crop-rotations from 0.26 to 0.26 ice y.l 3.1-6.6 tillage system in 0.24-0.43% at subsurface layer of soil. Effect of conventional and zero tillage on viable count of microbes under different crop rotations

study, DHA was affected with different tillage practices under different cropping systems and decreased with depth. DHA activity of different soil samples ranged between 51.4 to 64.4 and 44.7 to 55.5 $\mu\text{g TPF/g soil/24 h}$ under the conventional tillage system and increased in a range from 55.2 to 66.8 and 47.2 to 59.0 $\mu\text{g TPF/g soil/24 h}$ at surface and subsurface soil in zero-tillage system, respectively (Figure 2). Individually and interaction of crop-rotation, tillage and depth was significant. Our results are similar with the findings of Janušauskaite *et al.* (2013) that the highest dehydrogenase activity at a depth of 0-10 cm under no-tillage may be attributed to high NT microbial biomass and nutrients than CT. Similarly, Majchrzak *et al.*, 2016 and Bhaduri *et al.*, 2017 observed substantial effect of tillage systems on DHA, which was substantially higher under NT due to accumulation of organic matter and nutrients at the surface layer of soil under no-tillage.

During present investigation, long term impact of conservational tillage affected the alkaline phosphatase activity in soil. Upon the adoption of zero tillage system, alkaline phosphatase activity was relatively higher under rice-wheat cropping system 37 and 31 $\mu\text{g PNP/g soil/24 h}$ surfaces and subsurface layer, respectively (Figure 2). Individually and interaction of crop-rotation, tillage and depth was significant. The explanation for increased alkaline phosphatase activity in the surface layer may be due to high concentrations of inorganic phosphate released from organic matter

under zero tillage, resulting in increased alkaline phosphatase activity in the surface layer, the results are supported by the findings of that Acosta-martinez *et al.*, 2003 and Gajda *et al.*, 2013.

In present study, cellulase activity in soil under different tillage practices is shown in Figure 2. Cellulase activity was found higher in rice-wheat cropping pattern at 0-15 cm depth (0.058 $\mu\text{g glucose/g soil/24 h}$) and (0.049 $\mu\text{g glucose/g soil/24 h}$) at 15-30 cm depth under zero-tillage and guar-wheat cropping pattern had lower enzymatic activity at surface and subsurface layer 0.048 $\mu\text{g glucose g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ and 0.041 $\mu\text{g glucose/g soil/24 h}$, respectively, with same tillage and the similar trend was observed under conventional tillage. Individually, the effect of tillage and depth was significant at all locations. The findings of present study on relatively higher cellulase activity under ZT system are contrary to the observation of Bini *et al.* (2014) that no-tillage cellulase production was lower than traditional tillage due to increased organic matter inputs into the soil due to CT plowing and harrowing, which made organic C pools more vulnerable to microbial and soil enzyme attacks. However, Balota *et al.* (2003) reported 90% increase in cellulase activity under no-tillage as compared with CT in wheat-based cropping systems may be due to high microbial biomass in no-till soils which contained more nutrients than microbial biomass in CT systems.

In present study, urease activity was relatively higher in rice-wheat cropping system at surface soil

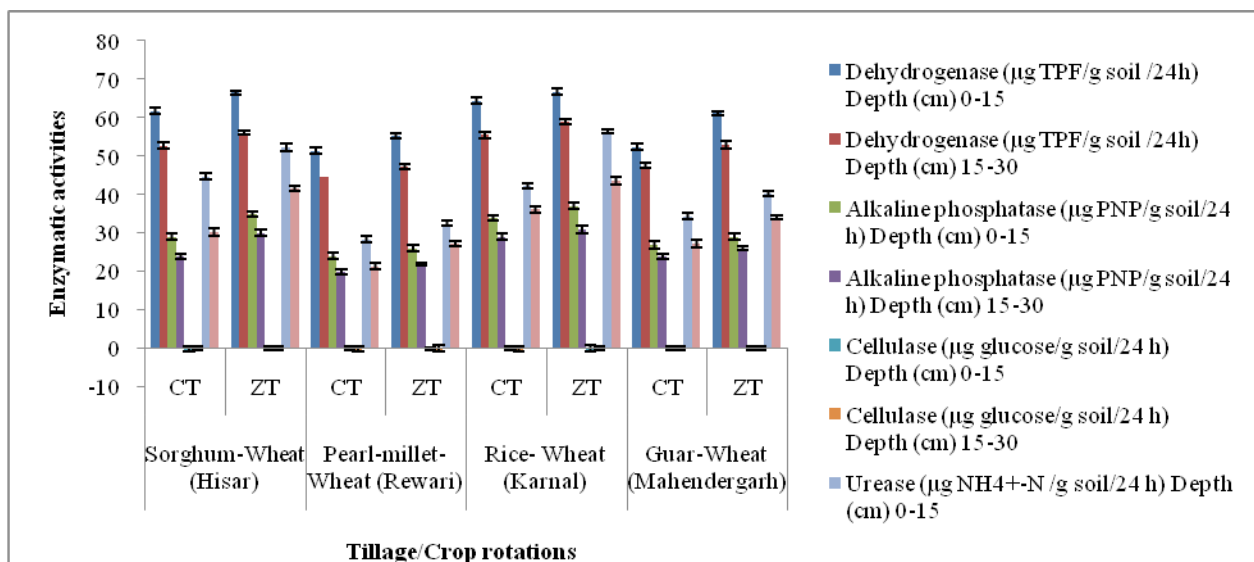


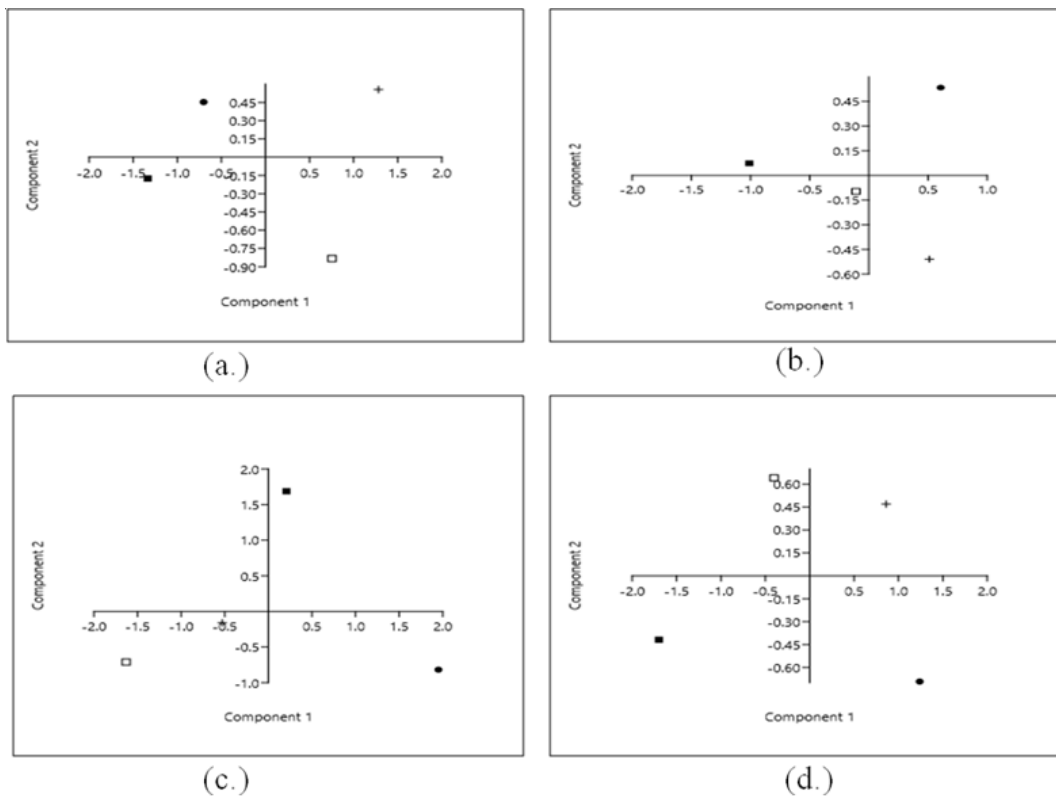
Fig. 2. Crop-rotations from 0.26 to 0.26 ice y.l 3.1-6.6 illage system in .24-0.43% at subsurface layer of soil. Effect of conventional and zero tillage on different enzymatic activities under different crop rotations

(56.4 $\mu\text{g NH}_4^+\text{-N}$ released /g soil/ 24 h) which further decreased in subsurface soil (43.6 $\mu\text{g NH}_4^+\text{-N}$ released /g soil/ 24 h) under zero tillage but with conventional tillage system respective values were (322.4 $\mu\text{g NH}_4^+\text{-N}$ released /g soil/ 24 h) and (36.2 $\mu\text{g NH}_4^+\text{-N}$ released /g soil/ 24 h) the similar trend was observed in other crop-rotations under conventional tillage (Figure 2). The findings of the present study on urease activity under various systems are similar to the observations of Raiesi and Kabiri (2016) that higher urease activity recorded under reduced tillage practices compared to CT practices may be due to high soil organic carbon accumulation under no-tillage. In comparison, Asenso *et al.* (2018) found the highest enzyme activity under subsoiling (SS) treatment relative to ZT treatment since SS treatment loosened the soil and resulted in higher organic C added to the soil, increasing the abundance of soil microorganisms resulting in increased soil enzyme activity due to increased substratum and oxygen availability.

Effect of tillage on functional diversity of microorganisms: Community-level physiological

profiling assesses the microbial community on the basis of sugar and amino acid utilization patterns and capacity to metabolize specific sole carbon sources. EcoPlate™ method can be used to study the variability of the community-level physiological profiling of microorganisms. During the present investigation, the results of Biolog® Ecoplate™ from different tillage practices with different crop-rotations revealed that the microbial community was relatively higher at surface layer under ZT in all cropping systems (Figure 3a-d).

Similar findings were reported by Habig and Swanepoel, (2015) that microbial diversity and activity were higher at surface layer under no-till than conventional tillage because the stimulation of soil microbial populations in no-tillage, promoted the availability of carbon sources for microbial utilization. Nivelles *et al.* (2016) found lowest AWCD and Shannon index under bare fallow and highest under cover crop-NT plots might be due to higher total nitrogen content and total organic carbon content that led to increased the diversity of substrate-richness and induced more microbial



*Dot means CT 0-15, Plus means CT 15-30, Square means ZT 0-15 and fill square means ZT 15-130

Fig. 3. Principal component analysis of conventional and zero tillage for (a.) Sorghum-wheat crop rotation at Hisar (b.) pearl millet-wheat crop rotation at Rewari (c.) Rice-wheat crop rotation at Karnal (d.) Guar-wheat crop rotation at Mahendragarh

enzymes because of greater metabolism of phenolic compounds and carbohydrates (under no-till) and polymers (under conventional till) as carbon sources in plots under standard cover crop. In contrary to our results, Janušauskaite *et al.* (2013) found higher AWCD values under conventional tillage than no-tillage because higher availability of hydrocarbon sources in conventional tillage could promote microbial community's diversity and increased use of carbon sources.

During present investigation, different soil samples under different crop-rotations were found related to each other, based on C source utilization pattern on principal component analysis. Galazka *et al.* (2017) observed that principal component analysis showed strong correlation between soil quality parameters and biodiversity indicators that explained 71.51 % biological variability in no-tilled soils.

CONCLUSION

Zero-tillage practice resulted in relatively higher chemical and microbiological properties at the surface layer, as well as changes in the soil microbial community and the tillage effect on microbial community varied by soil depths. The use of community level physiological profiling allows us to have better understanding regarding the changes of the microbial community under different management systems and might provide insights into how conservation tillage practice improves soil quality and sustainability.

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